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(54) Title: DELIVERY OF NUCLEIC ACIDS, PROTEINS, AND SMALL MOLECULES IN VITREOUS VESICULAR BODIES

(57) **Abrégé/Abstract:**

The present invention relates to compositions of aqueous humor and/or vitreous humor derived extracellular vesicles and their use for the delivery of therapeutic agents to ocular tissues for the treatment of ophthalmic diseases. Further disclosed are methods of making the compositions. Methods of treating and diagnosing an ocular condition are also disclosed.

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(54) Title: DELIVERY OF NUCLEIC ACIDS, PROTEINS AND SMALL MOLECULES IN VITREOUS VESICULAR BODIES

(57) Abstract: The present invention relates to compositions of aqueous humor and/or vitreous humor derived extracellular vesicles and their use for the delivery of therapeutic agents to ocular tissues for the treatment of ophthalmic diseases. Further disclosed are methods of making the compositions. Methods of treating and diagnosing an ocular condition are also disclosed.

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DELIVERY OF NUCLEIC ACIDS, PROTEINS, AND SMALL MOLECULES IN VITREOUS VESICULAR BODIES

[0001] This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/385,711 filed September 9, 2016, which is hereby incorporated by reference in its entirety.

[0002] This invention was made with government support under grant number UL1 TR000457-06 from the National Institutes of Health. The government has certain rights in this invention.

FIELD OF THE INVENTION

[0003] The present invention relates to methods and compositions for delivery of therapeutic agents to ocular tissues for the treatment of ophthalmic diseases.

BACKGROUND OF THE INVENTION

[0004] Therapies involving the delivery of nucleic acids (such as genes, mRNA, DNA, siRNA, miRNA, or other noncoding RNA), proteins, and/or small molecules to the intraocular structures have tremendous therapeutic potential in disease, including ocular disease. However, the inability to deliver biologically active molecules directly to their target site is a major limitation in treatment of eye disease. The blood-retinal barrier prevents penetration of most molecules into the retina. Similar limitations exist for other ocular tissues.

[0005] For example, a number of retinal degenerative conditions are due to single gene mutations. Delivery of a normal copy of the mutated gene or the protein encoded by the gene, has the potential to prevent progression of such diseases. Direct delivery of these genes is limited by a number of factors including instability of free genetic material in the extracellular milieu. Direct delivery of proteins can likewise be limited by instability in the extracellular tissues, as well limitations to penetration of the blood-retinal and other natural barriers. Development of a process to bring nucleic acids, proteins or small molecules into cells could transform ocular therapeutics.

[0006] Similarly, a number of retinal diseases such as wet age-related macular degeneration, diabetic retinopathy, macular edema from a number of causes, and others have been linked to elevated vascular endothelial growth factor (VEGF) levels. Intravitreal injection of antibodies or small molecules that inhibit VEGF is an effective therapy for these diseases; however, frequent injections are often required. A number of approaches have been attempted to reduce VEGF through alternative approaches, such as gene therapy using genes encoding naturally occurring anti-VEGF proteins such as sFLT-1. These gene therapy approaches have historically used viral

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vectors such as adenoviral vectors (or adeno-associated viral (AAV) vectors) for gene delivery. AAV vector-based delivery of genes for retinal disease is limited due to the potential toxicity or immunogenicity of the viral vector itself. Moreover, recent clinical trial attempted to utilize this technology and the trials were stopped due to failure.

5 [0007] The present invention is directed at overcoming this and other deficiencies of the art.

SUMMARY OF THE INVENTION

10 [0008] A first aspect of the present invention is directed to a composition comprising one or more aqueous humor and/or vitreous humor extracellular vesicular bodies. The aqueous humor and vitreous vesicular bodies of the composition are modified to contain one or more exogenous agents.

15 [0009] Another aspect of the present invention is directed to a method of delivering a therapeutic agent to select cells or tissue of a subject. This method involves providing a composition comprising one or more aqueous humor and/or vitreous humor vesicular bodies, where the vesicular bodies of the composition are modified to contain one or more therapeutic agent(s). The method further involves administering the composition to the subject under conditions effective to deliver the composition comprising the one or more aqueous humor and/or vitreous humor extracellular vesicular bodies modified to contain the therapeutic agent(s) to the select cells or tissue of the subject.

20 [0010] Another aspect of the present invention is directed to a method of making a composition comprising one or more aqueous humor and/or vitreous humor vesicular bodies, where the vesicular bodies of the composition are modified to contain one or more exogenous agents. This method involves providing a mammalian ocular fluid sample comprising vitreous and/or aqueous humor fluids, and isolating vesicular bodies from said ocular fluid sample. The method further involves inserting the one or more exogenous agents into the isolated vesicular bodies.

25 [0011] Described here in are compositions and methods of using vesicular bodies present in the vitreous humor and/or aqueous humor of the eye to deliver genes, proteins, or small molecules for therapeutic purposes. These vesicular bodies can be safely collected from the eye, emptied of their natural contents, and then filled with therapeutic substance (nucleic acid, protein, or small molecule). They can then be administered to the patient through a number of routes including intravenously or through intraocular injection. The vesicular bodies are taken up by the target cell, and the payload is released in a form suitable to exert therapeutic effects. Targeting molecules on the cell surface of the vesicular bodies can be modified to allow targeting of the vesicular body directly to the site of disease, thereby reducing toxicity to

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bystander tissues. Most importantly, because these vesicular bodies are endogenous, physiologic bodies that are already present in the vitreous, their harvesting, loading, and re-administration for therapeutic purposes can be performed with little toxicity or immunogenicity to the delicate neural structures of the eye.

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BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIGs. 1A-1G show extracellular vesicles (EV) escape from formalin-fixed bovine vitreous tissues and are retained with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC)-formalin fixation. FIG. 1A is a schematic diagram showing formalin-fixed vitreous (Vit) tissue immersed in wash buffer (supernatant) and heated to 37°C results in escape of EVs (arrowhead) and vitreous collagen (C, closed arrow) into the supernatant. FIGs. 1B-1C are representative transmission electron microscopy (TEM) photomicrographs of supernatant collected from formalin-fixed bovine vitreous tissue after incubation at 37°C and uranyl acetate (UA) and lead citrate staining show evidence of collagen strands (C, closed arrow) and numerous EVs (arrowhead) that are lost to the wash buffer. FIG. 1D is a schematic diagram showing EDC-formalin-fixed vitreous tissue immersed in wash buffer and heated to 37°C resulted in retention of EVs in the tissue, with no loss of EVs and minimal loss of vitreous collagen strands into the supernatant. FIG. 1E shows representative TEM photomicrographs of supernatant from EDC-formalin-fixed vitreous tissue after incubation at 37°C and UA and lead citrate staining showing few collagen strands (C, closed arrow) and no EVs in the supernatant. FIG. 1F shows representative TEM photographs of specificity control, PBS alone, which shows no collagen fibers nor EVs in the supernatant, but does show non-specific punctate staining of electron dense foci measuring less than 20 nm (NS, open arrow). FIG. 1G shows a western blot detecting exosome marker TSG-101 in supernatant (wash buffer) of formalin-fixed vitreous tissue (left lane) and vitreous sample (right lane). Scale bars are (FIG. 1B) 2.5 µm, (FIG. 1C), 500 nm, (FIG. 1E-1F), and 200 nm.

[0013] FIGs. 2A-2F show EDC-formalin fixation of bovine vitreous retains EVs imaged by multifocal microscopy (MPM), when compared to formalin fixation alone. FIG. 2A is a gross image of bovine vitreous placed on a vision testing card that demonstrates the highly transparent, gel-like structure. FIG. 2B shows representative MPM photomicrographs of whole mount bovine vitreous specimens fixed with formalin alone and stained with CFSE to mark protein (orange) and Hoechst to mark nuclei (purple). CFSE signal is observed surrounding the nuclei (FIG. 2B, left panel, open arrow), but not in the extracellular space. Nuclei staining shows no extracellular signal (FIG. 2B, left panel, purple, open arrow). FIG. 2C shows representative MPM photomicrographs of EDC-formalin-fixed vitreous stained with CFSE (orange) and

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Hoechst (purple). Overlay of image shows positive signal consistent with cell bodies (denoted with open arrow) and foci of extracellular protein signal (arrowheads) consistent in size and shape with EVs. FIG. 2D is an inset of FIG. 2C (white box), which shows multiple round intracellular foci (FIG. 2D, left panel, open arrowhead, orange) surrounding the area of nuclear stains (FIG. 2D, right panel, open arrowhead, purple). Numerous focal extracellular protein signals are observed (FIG. 2D, left panel, closed arrowheads, orange), consistent in size and shape with EVs, and no extracellular DNA is observed. FIG. 2E is a graph representing the mean $\pm$ standard deviation number of EVs per vitreous cell and shows that EDC-formalin-fixed vitreous exhibit significantly more EVs than formalin-fixed vitreous. FIG. 2F is a graphical representation of frequency distribution of bovine vitreous EV diameter imaged by MPM. EV sizes was measured for 4,000 EVs and the frequency of EVs were plotted against the diameter of the EV. The lower limit of multiphoton microscopy is 200 nm and EVs up to 6000 nm were measured. EVs were distinguished from cells and defined as containing extracellular protein or RNA without extracellular DNA. p-values are < 0.05 . Scale bars are (FIG. 2A) 1 cm, (FIG. 2B) 40 μ m, (FIG. 2C) 50 μ m and (FIG. 2D) 10 μ m.

[0014] FIGs. 3A-3C show fixation of bovine vitreous with EDC-formalin retains EVs and extracellular RNA *in situ*. FIGs. 3A-3C shows representative confocal fluorescent photomicrographs of whole mount bovine vitreous specimens crosslinked with EDC-formalin (FIGs. 3A and 3B) or formalin alone (FIG. 3C), stained with propidium iodide (PI, red) to mark DNA and RNA, Hoechst (blue) to visualize DNA and nuclei, and carboxyfluorescein succinimidyl ester (CFSE, green) to stain for protein. FIG. 3A is an overlay of images from EDC-formalin-fixed bovine vitreous and shows positive signal consistent with cell bodies (FIG. 3A, denoted with open arrow) and foci of extracellular RNA (closed arrowhead) and extracellular protein (closed arrowhead) consistent in size and shape with EVs. FIG. 3B shows representative confocal fluorescent photomicrographs of EDC-formalin-fixed vitreous and shows multiple round cellular foci (FIG. 3B, all panels, open arrowhead) and numerous focal signals of extracellular RNA (FIG. 3B, left panel, PI stain, red) and extracellular protein (FIG. 3B, right panel, CFSE stain, green) between the cells. FIG. 3C shows representative photomicrographs of whole mount bovine vitreous fixed with formalin alone and shows signal for RNA (FIG. 3C, left panel, PI, red) in the nucleus, similar to nuclei staining (FIG. 3C, middle panel, Hoechst, blue). Formalin-only fixed vitreous show no foci of extracellular RNA signal (FIG. 3C, left panel). CFSE stain shows cellular signal (open arrow), but no EV-shaped extracellular protein signal (FIG. 3C, right panel, green, no punctate staining observed between open arrows). The cell size appears smaller in the formalin only fixation, presumably due to EDC-formalin retaining more

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cytoplasmic RNAs and protein as compared to formalin alone. Scale bars are (FIG. 3A) 25 μ m and (FIGs. 3B-3C) 50 μ m.

[0015] FIGs. 4A-4C show RNase treatment of EDC-formalin-fixed bovine vitreous stained with PI show reduced extracellular signal. FIG. 4A shows low-power wide-field fluorescent photomicrographs of whole mount bovine vitreous specimens crosslinked with EDC-formalin and stained with PI (FIG. 4A, top panel, red) and shows signal in the extracellular environment of vitreous tissue (denoted with closed arrowhead, inset), nuclei labeled (FIG. 4A, middle panel, Hoechst, blue) and merged images are shown (FIG. 4A, bottom panel). Vitreous cell nuclei stain positive with PI and Hoechst; colocalized signals are shown in green (FIG. 4A, bottom panel, inset). Cells are denoted with an open arrow and foci of extracellular PI signal are marked with a closed arrowhead (FIG. 4A, top and middle panel, inset). Nuclei were stained, and no extracellular DNA signal is observed (FIG. 4A, bottom panel). FIG. 4B shows photomicrographs of whole mount bovine vitreous fixed with EDC-formalin and treated with RNase A. Samples stained with PI (FIG. 4B, top panel, red), Hoechst (FIG. 4B, middle panel), and merged images are shown (FIG. 4B, bottom panel). RNase A treated samples show no evidence of extracellular RNAs as demonstrated by the lack of signal between the cell bodies (FIG. 4B, top and middle panel) and show no signal between two cell nuclei (open arrows). The PI signal for cytoplasmic RNA in RNase A treated samples (FIG. 4B, top panel) appear smaller than pre-RNase treated samples (FIG. 4A, top panel), presumably due to EDC-formalin retaining more cytoplasmic RNA. FIG. 4C is a graphical representation of mean $\pm$ standard deviation foci of extracellular signal for EDC-formalin fixed tissues stained with PI pre-RNase treatment and after RNase treatment show significantly fewer EVs after RNase treatment. Mean $\pm$ standard error for EVs per cell were 60.7 $\pm$ 35.1 pre-RNase treatment and 0.03 $\pm$ 0.04 post RNase treatment, with significantly more EVs per cell noted pre-RNase treatment ($p < 0.001$) (FIGs. 4C). Scale bars are (FIGs. 4A-4B) 50 μ m and (4A inset, 4B inset) 20 μ m.

[0016] FIGs. 5A-5B show EDC-formalin fixation of bovine vitreous retains EVs imaged with photomicroscopy. FIGs. 5A-5B show low-power wide field fluorescent photomicrographs of whole mount bovine vitreous specimens crosslinked with EDC-formalin (FIG. 5A) or formalin alone (FIG. 5B). FIG. 5A shows representative photomicrographs of bovine vitreous fixed with EDC-formalin and stained with CFSE to label protein (FIG. 5A, top and middle panel, white) and Hoechst to label nuclei (FIG. 5A, bottom panel, blue) and shows multiple round cellular foci (FIG. 5A, all panels, open arrowhead) with numerous extracellular protein signals (top and middle panels, CFSE, white) consistent with EVs. FIG. 5B shows photomicrographs of whole mount bovine vitreous fixed with formalin only show nuclear stain (FIG. 5B, middle and bottom panels, Hoechst, blue) co-localizing with CFSE (FIG. 5B, top and middle panel, white),

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consistent with cellular DNA and nucleic acid, respectively. There is no evidence of extracellular protein signal (FIG. 5B, top and middle panel, CSFE, white). The CFSE stained cell size appears smaller in the formalin only fixation (FIG. 5B, middle panel) as compared to EDC-formalin fixation (FIG. 5A, middle panel), presumably due to EDC-formalin retaining
 5 more small protein as compared to formalin fixation alone. Scale bars are (FIGs. 5A-5B) 100 μ m.

[0017] FIGs. 6A-6I show bovine and human vitreous humor contains EVs. FIG. 6A shows representative transmission electron microscopy (TEM) photomicrographs of bovine vitreous tissue sections stained with uranyl acetate (UA) and lead citrate and shows a substantial number
 10 of EVs that are pleomorphic in size (arrowheads) and that contact collagen strands (marked with a "C" and arrow). The inset (upper right corner) is an enlargement of the area-enclosed box in the lower right corner and shows an EV associated with a collagen strand. FIG. 6B shows representative TEM photomicrograph of EVs isolated from bovine vitreous and stained with the electron dense protein stain, CSFE, which depict EV morphology and show numerous EVs
 15 pleomorphic in size (smaller EV marked with arrowhead, larger EV with double arrowhead). FIG. 6C shows representative TEM photomicrograph of EVs isolated from bovine vitreous and electron dense nucleic acid stain acridine orange (AO) staining and shows large EVs (double arrowhead) positive nucleic acid signal. FIG. 6D shows multiple EVs (arrowheads) in a network of collagen within whole mounted bovine vitreous stained with ethidium bromide (EtBr), an
 20 electron dense and nucleic acid stain. FIG. 6E shows a graphical representation of the mean (black line) $\pm$ standard error (red bars) concentration EVs according to EV diameter, based on nanoparticle tracking analysis of EVs isolated from bovine vitreous. FIG. 6F shows representative TEM photomicrographs of postmortem human eye sections stained with UA and lead citrate show a substantial number of EVs at the vitreous base (Vit), adjacent to the non-
 25 pigmented epithelium (NPE) of the ciliary body (smaller EVs marked with arrowhead, larger EVs with double arrowhead). The EVs (FIGs. 6F-6G, arrowheads) contact with collagen strands (arrows). FIG. 6H shows representative TEM photomicrographs of EVs isolated from human vitreous and stained with AO show EVs (arrowhead) with positive nucleic acid signal. FIG. 6I is a graphical representation of frequency distribution of human vitreous EV diameter. Scale bars
 30 are (FIG. 6A, FIG. 6G) 100 nm, (FIG. 6B) 50 nm, (FIG. 6C-6D, FIG. 6H) 200 nm, and (FIG. 6F) 2 μ m.

[0018] FIGs. 7A-7D show immunohistochemistry staining of EV-specific protein TGS-101 in normal bovine vitreous. FIG. 7A shows representative wide-field fluorescent photomicrographs of whole mount bovine vitreous specimens fixed with formalin and processed
 35 at cold temperatures and demonstrates immunohistochemical stain for the EV-associated protein,

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TGS-101, in the extracellular space (FIG. 7A, top and middle panels, arrowhead, Alexa 488, Green). The inset (FIG 7A, all panels, top right) is a higher magnification image of the box in the middle (FIG. 7A, all panels). Nuclei are marked with Hoechst counterstain (FIG. 7A, top and bottom, blue, open arrow). Hundreds of punctate extracellular signals were observed (FIG. 7A, top and middle). No evidence of extracellular DNA was observed (FIG. 7A, bottom). FIG. 7B shows representative photomicrographs from specificity controls for TSG-101 immunohistochemistry: whole mount normal bovine vitreous labeled with non-specific IgG antibody (green). The inset (FIG. 7B, all panels, top right) is a higher magnification image of the box in the middle (FIG. 7B, all panels). Signal was observed surrounding the nuclei (FIG. 7B, top and middle, Alexa 488, green). Images show no evidence of extracellular signal (FIG. 7B, top and bottom, Hoechst, blue). FIG. 7C is a graphical representation of mean $\pm$ standard error for TSG-101 signal in extracellular and intracellular spaces, $*p < 0.05$ by Student's unpaired t-tests. FIG. 7D shows positive signal for TSG-101 is observed in the extracellular space of the formalin-fixed vitreous (FIG. 7D, left, green). Nuclei are labeled with Hoechst (FIG. 7D, left, blue) and PI (FIG. 7D, right, red). There is no evidence of extracellular RNA in formalin-fixed samples (FIG. 7D, right, red). Scale bars are (FIGs. 7A-7B) 40 μ m and (FIG. 7A inset, FIG. 7B inset and FIG. 7D) 10 μ m.

[0019] FIGs. 8A-8B show bovine vitreous is free of cells after low-speed centrifugation. FIG. 8A shows representative low power light microscopy photomicrographs of whole mount bovine vitreous after low-speed centrifugation followed by hematoxylin and eosin staining. These images show eosinophilic signal consistent with vitreous collagen (pink, arrow) without evidence of hematoxylin stained cellular nuclei. FIG. 8B shows images of whole mount vitreous prior to centrifugation. These images show eosinophilic signal consistent with vitreous collagen (pink, arrow) with evidence of hematoxylin stained cellular nuclei (purple, open arrow). Scale bars are (FIGs. 8A-8B) 50 μ m.

[0020] FIGs. 9A-9I show human and bovine vitreous EV transfer endogenous RNA into cultured cells. FIGs. 9A-9C show representative confocal photomicrograph images of a human retinal pigment epithelial cells (ARPE-19) after 24 h treatment with a bolus of bovine vitreous EVs that were pre-labeled with the nucleic acid stain acridine orange (AO). Images show uptake of EV-labeled RNA in ARPE-19 cells (FIG. 9A, green). Nuclei are labeled (FIG. 9B, Hoechst, purple) and a merged image (FIG. 9C) shows transfection of ARPE-19 cells, with AO signal in the cytoplasm. FIG. 9D is a graphical representation of transfection efficiency (% of cells transfected) for ARPE-19 cells treated with bovine vitreous EVs (error bars represent standard deviation, $n = 3$, $p < 0.05$). FIGs. 9E-9F show representative confocal photomicrographs of human embryonic kidney (HEK) cells treated with a 24 h bolus of bovine EVs bodies pre-

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labeled with AO and show staining in the cytoplasm (FIG. 9E). Nuclei were labeled and a merged image is shown (FIG. 9F). FIGs. 9G and 9H are representative low-power fluorescent photomicrograph images of ARPE-19 cells treated for 3 h with a bolus of EVs that were isolated from post-mortem human vitreous and pre-labeled with AO. The image of FIG. 9G shows

5 transfection of cells (FIG. 9G, AO, green). Nuclei were marked (FIG. 9H, Hoechst, blue). FIG. 9I is a graphical representation of transfection efficiency (% of cells transfected) for ARPE-19 cells treated with human vitreous EVs (error bars represent standard deviation, $n = 3$, $p < 0.05$). Scale bars are (FIGs. 9A-9C) 50 μm , (FIGs. 9E-9F) 15 μm , and (FIGs. 9G-9H) 100 μm .

[0021] FIGs. 10A-10F show delivery of recombinant bovine serum albumin (BSA) protein and recombinant green fluorescent protein (GFP) by bovine vitreous extracellular vesicles (EV) to cultured human retinal pigment epithelial (ARPE-19) cells. FIG. 10A are representative photomicrographs of ARPE-19 cells treated with a bolus of bovine vitreous EVs that had been pre-loaded with 1 μg BSA conjugated to fluorescein by electroporation at 300 V. The left image of FIG. 10A shows fluorescein staining (yellow) in the cytoplasm. FIG 10A, middle image

15 shows nuclei labelled with Hoechst stain (blue), and a merged image (FIG. 10A, right) shows substantial number of cells transfected. FIG. 10B are representative photomicrographs of ARPE-19 cells treated with a bolus of bovine vitreous EVs that had been mixed with BSA-fluorescein without electroporation (0 V, control). FIG. 10B, left image show no fluorescein staining, while FIG. 10B, right image shows nuclei labeling with Hoechst stain (blue). FIG. 10C

20 is a graphical representation of mean $\pm$ standard deviation transfection efficiency (% of cells transfected) of ARPE-19 cells treated with vitreous EVs loaded with 3 μg , 1 μg , or 0.5 μg BSA-fluorescein by electroporation at 300 V, with EVs loaded with 0.5 μg BSA-fluorescein without electroporation (0 V, control), or with PBS alone without electroporation (0 V, control). $p < 0.001$ for all BSA-fluorescein dosages loaded at 300 V vs. controls at 0 V. FIG. 10D shows

25 representative photomicrographs of ARPE-19 cells after application of a bolus of bovine vitreous EVs that had been pre-loaded with 1 μg of recombinant GFP by electroporation at 300 V. FIG. 10D, left image, shows positive GFP staining (green) in the cytoplasm. FIG. 10D, middle image, shows nuclei labelled with Hoechst stain (blue), and a merged image (FIG. 10D, right) shows substantial number of cells transfected. FIG. 10E, right image, shows no fluorescein staining in

30 a representative photomicrograph of ARPE-19 cells after application of a bolus of bovine vitreous EVs that had been mixed with GFP without electroporation (0 V, control). Nuclei labeling with Hoechst stain (blue) in the control sample is shown FIG. 10E, right image. FIG. 10F is a graphical representation of mean $\pm$ standard deviation transfection efficiency (% cells transfected) of ARPE-19 cells after application of EVs loaded with 1 μg , 0.5 μg , or 0.25 μg GFP

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by electroporation at 300 V or 1 µg GFP without electroporation (0 V, control). $p < 0.05$ for all GFP dosages loaded at 300 V vs. control at 0 V. Scale bars (FIGs. 10A-10E) 50 µm.

[0022] FIGs. 11A-11D show bovine vitreous EVs target the retina and deliver recombinant protein *in vivo*. FIG. 11A are representative wide-field fluorescent photomicrographs of mouse retina tissue sections after injection of a dilute amount of bovine EVs loaded with recombinant bovine serum albumin (BSA) conjugated to fluorescein on day 3 post injection. FIG. 11A, left image shows BSA fluorescein only, FIG. 11A, middle image, shows nuclei staining with Hoeschst only, and FIG 11A, right image, shows a merged image. The images of FIG. 11A show signal in vitreous that does not penetrate the inner limiting membrane (ILM). FIG. 11B are representative confocal photomicrographs of mouse retina tissues section 3 weeks after injection of BSA-fluorescein showing expression in the retinal outer plexiform layer (OPL) and inner plexiform layer (IPL, arrow). FIG. 11B, left image, shows BSA fluorescein only, FIG. 11B, middle image, shows nuclei staining only, and FIG 11B, right image, shows a merged image. FIG. 11C are images showing signal in cells traversing the IPL and OPL, as well as, ganglion cells (marked with inset box). The inset box from (FIG. 11C) is shown in higher power in (FIG. 11D) demonstrating positive stain in a cluster of cells in ganglion cell layer (GCL) and retinal nerve fiber layer. FIGs. 11C-D, left image, shows BSA fluorescein only, FIGs. 11C-D, middle, image, show nuclei staining only, and FIGs. 11C-D, right images, show a merged view. Scale bars are 30 µm (FIG. 11A), 50 µm (FIGs. 11B-11C) and 25 µm (FIG. 11D). Photoreceptor segments (ph segments), outer nuclear layer (ONL), inner nuclear layer (ONL).

[0023] FIGs. 12A-12E show bovine vitreous EVs target the cornea, ciliary body, and retina to deliver recombinant protein *in vivo*. FIG. 12A are representative confocal fluorescent photomicrographs of mouse eye tissue sections after injection of bovine EVs loaded by electroporation (300 V) with recombinant bovine serum albumin (BSA) conjugated to fluorescein (BSA-fluorescein) at 3-weeks post injection showing signal in cornea from endothelial cells and corneal keratocytes (FIG. 12A, left image shows BSA fluorescein only, FIG. 12A, middle image, shows nuclei staining with Hoeschst only, and FIG 12A, right image, shows a merged image). FIG. 12B are images from control group of bovine EV mixed with BSA-fluorescein without electroporation (0 V) after 3-week injection showin no expression in endothelial cells nor corneal keratocytes, but does show non-specific staining of the corneal epithelium (FIG. 12B, left image shows BSA fluorescein only, FIG. 12B, middle image, shows nuclei staining with Hoeschst only, and FIG 12B, right image, shows a merged image). FIG. 12C are representative confocal fluorescent photomicrographs from mouse eyes at 3-week post injection of EVs loaded by electroporation (300 V) with BSA-fluorescein that show signal in non-pigmented ciliary epithelial cells (FIG. 12A, left image shows BSA fluorescein only, FIG.

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12C, middle image, shows nuclei staining with Hoeschst only, and FIG 12C, right image, shows a merged image). FIG. 12D are images showing robust expression of BSA-Fluorescein in the photoreceptors, inner plexiform layer (IPL), retinal pigment epithelial (RPE) cells, and choroid (FIG. 12D, left image shows BSA fluorescein only, FIG. 12D, middle image, shows nuclei staining with Hoeschst only, and FIG 12D, right image, shows a merged image). FIG. 12E are images of the mouse retina photoreceptors and retinal pigment epithelium (RPE) that are transfected with recombinant BSA protein that was delivered by EVs. Scale bars are 25 μ m (FIGs. 12A-12E). Corneal epithelium (Epi), corneal endothelium (endo), outer plexiform layer (OPL), outer nuclear layer (ONL), inner plexiform layer (ONL).

10 **[0024]** FIGs. 13A-13I show bovine vitreous vesicular bodies loaded with fluorescent labeled siRNAs transfects into human retinal pigment epithelial cells with high efficiency. FIGs. 13A-13C are low-power fluorescent photomicrographs of human retinal pigment epithelial (ARPE-19) cells that show transfection of anti-GAPDH siRNA conjugated to cyanine 3 dye (siRNA-Cy3) after electroporation with bovine vesicular bodies at 350 V (FIG. 13A, yellow, Cy3), nuclei marked with Hoechst dye (FIG. 13B, blue), and merge image of FIG. 13A and FIG. 13B shows substantial number of cells transfected (FIG. 13C). FIGs. 13D-13F are low-power photomicrographs of ARPE-19 cells treated with bovine vitreous vesicular bodies containing siRNA-Cy3 after electroporation at 200 V. FIG. 13D shows siRNA-Cy3 staining in the cytoplasm (yellow). Nuclei were labeled with Hoescht stain (FIG. 13E, blue), and the merged image of FIG. 13F show staining in the cytoplasm with reduced cell staining when compared to 350 V. FIGs. 13G-13H are images showing ARPE-19 cells treated with a bolus of bovine vesicular bodies and anti-GAPDH siRNA-Cy3 without electroporation (0 V). FIG. 13G shows no siRNA-Cy3 staining in ARPE-19 cells, and FIG. 13H shows nuclei marked with Hoechst stain (blue). The graph of 13I shows the percent of cells transfected with siRNA-GAPDH-Cy3 by electroporation voltage. Scale bars are 50 μ m (FIGs. 13A-13H).

30 **[0025]** FIGs. 14A-14F show the bovine ciliary body non-pigmented epithelium produces abundant vesicular bodies and that are released into intracellular spaces. FIGs. 14A-14C are TEM photomicrograph images from bovine sections of ciliary body nonpigmented epithelium (NPE) stained with uranyl acetate showing multiple vesicular bodies (FIG. 14A, arrowheads) within the lumen of enlarged intercellular spaces (ISP) and budding from the NPE surface (FIG. 14A, asterisk). The orientation of the image is such that the base of the NPE and vitreous base marked (VIT) and internal limiting membrane (ILM) are shown. FIG. 14B is the inset from FIG. 14A, upper box, and shows vesicular bodies within ISP. FIG. 14C shows the lower inset from FIG. 14A and shows a NPE cell with a vesicular body budding into the lumen of the ISP (FIG. 14C, asterisk). FIG. 14D is a TEM photomicrograph of NPE showing electron dense bodies

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within the cell (FIG. 14D, wedge) and in vesicular bodies in the ISP lumen (FIG. 14D, arrowheads). FIG. 14E is a TEM photomicrograph of ciliary body pigmented epithelium (PE) showing no evidence of budding vesicles. FIG. 14F is a TEM image of bovine vitreous base attached to the ciliary body showing collagen fibers with several vitreous bodies (FIG. 14F, arrow-heads) within the collagen matrix (FIG. 14F, arrows). Scale bars are 1 μ m (FIG. 14A), 200 nm (FIG. 14B and FIG. 14F), 250 nm (FIG. 14C), and 500 μ m (FIGs. 14D-14E).

[0026] FIGs. 15A-15I show vitreous vesicular bodies loaded with exogenous protein transfects with high efficiency into human retinal pigment epithelial cells. FIGs. 15A-15C are low-power fluorescent photomicrographs of human retinal pigment epithelial (ARPE-19) cells showing uptake of exogenous bovine serum albumin. Bovine vitreous humor vesicular bodies were electroporated at 350 V with BSA-fluorescein and then a bolus given to ARPE-19 cells which showed substantial staining in the cytoplasm (FIG. 15A, yellow). Nuclei were labeled with Hoechst stain (FIG. 15B, blue), and a merged image of FIG. 15A and FIG. 15B shows a substantial number of ARPE-19 cells stain for fluorescein (FIG. 15C). ARPE-19 cells treated with bovine vitreous vesicular bodies containing BSA-fluorescein (electroporated at 200 V) also show staining in the cytoplasm (FIG. 15D). Nuclei of these cells were labeled with Hoechst stain (FIG. 15E, blue), and the merged images (FIG. 15F) shows a decrease in cytoplasm cytoplasmic staining, which means fewer transfected cells as compared to cells exposed to EV electroporated with 300 V. FIGs. 15G-15H are photomicrographs showing ARPE-19 cells treated with bovine vesicular bodies and BSA-fluorescein without electroporation. No BSA-fluorescein staining in ARPE-19 cells was observed as shown in FIG. 15G). Nuclei were marked with Hoechst stain (FIG. 15H, blue). FIG. 15I is a graph showing protein transfection efficiency (error bars standard deviation of measurements, $n = 3$). Scale bars are 50 μ m (FIGs. 15A-15H).

[0027] FIGs. 16A-16E show aqueous humor contains abundant vesicular bodies. FIG. 16A are TEM photomicrographs of whole mount bovine aqueous labeled with acridine orange showing multiple vesicular shaped bodies of various sizes (FIG. 16A, single arrowheads, double arrowheads, and arrow marks small, medium and large vesicle, respectively). FIG. 16B is a TEM photomicrograph depicting vesicular bodies stained with CFSE (FIG. 16B, arrowheads) associated with a collagen stand (FIG. 16B, arrow). FIGs. 16C-16D are TEM photomicrographs of bovine anterior chamber exosomes after isolation by differential ultracentrifugation stained with uranyl acetate (FIG. 16C) and acridine orange (FIG. 16D) showing a cluster of vesicular bodies of various sizes (FIG. 16C). FIG. 16E is an averaged Finite Track Length Adjustment Size/Concentration graph from nanoparticle tracking analysis of ultracentrifuge-isolated exosomes (error bars indicating ± 1 standard error of the mean). The data had a concentration

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of 1.10×10^8 , a mean of 140.8 nm, a standard deviation of 127.9 nm, and peaks at 15 nm, 35 nm, 65 nm, 115 nm, 185 nm, 205 nm, 225 nm, 275 nm, 415 nm, 505 nm, and 615 nm (FIG. 16E). Scale bars, 200 nm (FIG. 16A, FIGs. 16C-16D) 500 nm (FIG. 16B).

[0028] FIGs. 17A-17R show that isolated bovine EVs can transfect human skin cells.

5 Isolated bovine EVs were labeled for endogenous proteins (CFSE) and RNA (acridine orange) and then human skin cells (PAM-212) were transfected in culture. FIGs. 17A-17C are low power wide-field photomicrographs of human skin cells PAM-212 that were transfected with bovine vitreous RNA that was labeled with acridine orange (AO) after 3 h of transfection. FIGs. 17D-17F show PAM-212 cells transfected with AO labeled EVs 24 hours after transfection. 10 Images show a robust transfer of bovine EV RNA to human skin cells. The images of FIGs. 17G-17I show PAM-212 cells transfected bovine vitreous EVs that were previously labeled for all protein using CFSE, EVs were isolated again and then exposed to a bolus of EV-labeled protein to PAM 212 cells. After transfection of 3 h (FIGs. 17G-17I) and 24 hours (FIGs. 17J-17L) cells show robust uptake of bovine EV protein. Negative controls show no transfection at 15 3h (FIGs. 17M-17O) and 24 h (FIGs. 17P-17Q). Scale bar is 100 μ m for all images.

DETAILED DESCRIPTION OF THE INVENTION

[0029] The present invention is based on the unexpected discovery of a vesicular network in the vitreous and aqueous humor of the healthy human and bovine eyes. The vesicles of this network are loaded with a cargo of diverse proteins and coding and non-coding RNAs that they 20 transport short and long distances to other ocular tissues. As described and demonstrated herein, these vesicle bodies can be safely isolated from ocular fluids of healthy individuals and modified to serve as therapeutic delivery vehicles.

[0030] Accordingly, a first aspect of the present invention is directed to a composition comprising one or more aqueous humor and/or vitreous humor extracellular vesicle bodies. The 25 aqueous humor and/or vitreous extracellular vesicle bodies of the composition are modified to contain one or more exogenous agents.

[0031] The term “extracellular vesicle” as used herein refers to a nanosized membranous particle secreted by a cell. Extracellular vesicles, which are also referred to as EVs, multivesicular bodies, and ectosomes, are natural transport nanovesicles that have been 30 implicated in intercellular communication via transfer of biomolecules such as proteins, lipids, and RNA from one cell to another. Extracellular vesicles differ from other secreted vesicles, *e.g.*, exosomes and apoptotic bodies, based on their size, *i.e.*, exosomes are typically about 40-100 nm in diameter, extracellular vesicles are typically 100-1000 nm in size, and apoptotic bodies are typically 1-5 μ m in size.

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[0032] In accordance with the present disclosure, the extracellular vesicles of the vitreous and aqueous humor are characterized by their size, *i.e.*, their diameter. The term “diameter” refers to the maximum dimension of the vesicle, it being understood that the vesicle is not necessarily spherical. Vesicle diameter can be measured using conventional techniques for measuring nanoparticle size, such as microscopy techniques (*e.g.*, transmission electron microscopy or light scattering techniques). In another embodiment, the vesicle diameter is measured using Nanoparticle Tracking Analysis (*see* WO03/093801 to Carr and Geddes, which is hereby incorporated by reference in its entirety).

[0033] The vesicular bodies of the vitreous humor are heterogenous in size, having a diameter ranging from 100 nm to 6000 nm. In one embodiment, the extracellular vesicles of the composition derived from the vitreous humor have a diameter ranging from 100 nm to 1000 nm. In another embodiment, the extracellular vesicles of the composition derived from the vitreous humor have a diameter of about 150 to 500 nm. In another embodiment, the extracellular vesicles of the composition derived from the vitreous humor have a diameter of about 150 to 300 nm.

[0034] The vesicular bodies of the aqueous humor are also heterogenous in size and generally smaller than the vitreous vesicular bodies. In one embodiment, the extracellular vesicles of the composition derived from the aqueous humor have a diameter ranging from 50 nm to 600 nm. In another embodiment, the extracellular vesicles of the composition derived from the aqueous humor have a diameter of about 50-400 nm. In another embodiment, the extracellular vesicles of the composition derived from the aqueous humor have a diameter of about 50-200 nm.

[0035] In one embodiment, the composition comprising aqueous humor and/or vitreous humor extracellular vesicle bodies comprises a population of vesicle bodies. A “population” of vesicles refers to a set of at least 2 vesicle bodies, at least 5 vesicle bodies, at least 10 vesicle bodies, at least 50 vesicle bodies, at least 100 vesicle bodies, at least 500 vesicle bodies, at least 1000 vesicle bodies, at least 10000 vesicle bodies, at least 100,000 vesicle bodies, at least 1,000,000 vesicle bodies, or more.

[0036] In another embodiment, the vesicle bodies of the vitreous and aqueous humor are characterized by their proteomic signature. For example, the vesicles of the vitreous humor express several known exosome markers, including CD-9, Hsp-90 β , annexin-II, and TSG-101 proteins. The full list of exosome marker proteins present and enriched in vitreous extracellular vesicles is provided in Table 1 *infra*. In one embodiment, the composition comprises a population of vitreous humor extracellular vesicles expressing one or more exosome markers listed in Table 1. In another embodiment, the composition comprises a population of vitreous

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humor extracellular vesicles expressing two, three, four, five, six, seven, eight, nine, or all ten of the exosome markers listed in Table 1.

[0037] The vitreous humor vesicles of the composition of the present disclosure also possess a diverse proteomic signature of eye specific proteins as described in the Examples
5 herein, *see* Table 2 *infra*. In one embodiment, the composition comprises a population of vitreous humor extracellular vesicles expressing one or more of the eye specific proteins listed in Table 2. In another embodiment, the composition comprises a population of vitreous humor extracellular vesicles expressing two, three, four, five, six, seven, eight, nine, ten or all eleven of the eye specific proteins listed in Table 2.

10 [0038] The entire protein signature of the vitreous extracellular vesicles is provided in Table 3 *infra*. Table 3 indicates the differential expression of the listed protein between the extracellular vesicle fraction of the vitreous humor and the cell free fraction of the vitreous humor. Accordingly, in one embodiment, the composition described herein comprises a population of extracellular vesicles expressing one or more of the proteins listed in Table 3. IN
15 another embodiment, the composition described herein comprises a population of extracellular vesicles expressing one or more of the proteins enriched for in the extracellular fraction of the vitreous fraction. In another embodiment, the composition described herein comprises a population of extracellular vesicles expressing one or more of the proteins identified in Table 3 as being expressed only in the extracellular vesicle fraction. The population of extracellular
20 vesicles described herein can be defined by the expression of any combination of proteins identified as being differentially expressed in only the extracellular vesicle fraction.

[0039] In one embodiment, the aqueous humor and/or vitreous humor vesicular bodies of the composition are isolated vesicular bodies. As used herein, the term “isolated” refers to vesicular bodies that have been removed from a human or animal body, *i.e.*, from ocular fluids of
25 the animal or human, and substantially separated from cell or cellular debris with which they are normally associated *in vivo*. In one embodiment, the composition comprising the extracellular vesicles is >75%, >80%, >85%, >90%, >95% free of cell or cellular debris normally associated with said vesicle bodies *in vivo*.

[0040] The extracellular vesicles of the composition may be isolated and/or purified using
30 several techniques. These include filtration, centrifugation, ion-chromatography, or concentration, either alone or in combinations. An exemplary isolation method is described herein and involves a series of low-speed centrifugations. Other exemplary methods of isolating or purifying extracellular vesicles that are known in the art and suitable for use in accordance with the present invention include, without limitation, those disclosed by, *e.g.*, van der Pol et al.,
35 “Recent Developments in the Nomenclature, Presence, Isolation, Detection and Clinical Impact

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of Extracellular Vesicles,” *J Thromb Haemost* 14:48-56 (2016), U.S. Patent Application Publication No. 2016/0216253 to Balaj, WO2015/143113 to Cohen, WO2000/44389 to Dhellin, and WO2001/82958 to Lamparski, which are hereby incorporated by reference in their entirety.

[0041] In one embodiment, the composition as disclosed herein comprises extracellular
5 bodies from the vitreous humor. The vitreous humor or vitreous body is located between the lens and the retina. It is an optically clear, mostly acellular, and gel-like structure with little known biological function. In one embodiment, the extracellular vesicles are obtained from a healthy, normal vitreous body, *i.e.*, from a healthy subject. In another embodiment, the extracellular vesicles are obtained from a vitreous of a subject having an ocular disease. In
10 another embodiment, the composition comprises extracellular bodies from the aqueous humor. The aqueous humor is the clear liquid filling the anterior chamber of the eye, located between the lens and the cornea. In one embodiment, the extracellular vesicles are obtained from a healthy, normal aqueous humor, *i.e.*, from a healthy subject. In another embodiment, the extracellular vesicles are obtained from an aqueous humor of a subject having an ocular disease. In another
15 embodiment, the composition comprises a mixture of extracellular bodies obtained from the aqueous humor and the vitreous humor.

[0042] In one embodiment, the extracellular vesicles as described herein are secreted by the ciliary body, *e.g.*, the ciliary epithelium. In another embodiment, the extracellular vesicles as described herein are secreted by the pigmented ciliary epithelium, non-pigmented ciliary
20 epithelium, ciliary processes. In another embodiment, the extracellular vesicles as described herein are secreted by retinal cells including Müller cells, ganglion cells, amacrine cells, horizontal cells, photoreceptors (rods and cones) bipolar cells, retinal pigment epithelium or retinal endothelial cells. In another embodiment, the extracellular vesicles as described herein are secreted by cells of cornea including corneal epithelium, corneal stroma (keratocytes), corneal
25 endothelium, or limbal stem cells. In another embodiment, the extracellular vesicles as described herein are secreted by cells of iris including pigmented or non-pigmented cells, spindle shaped fibroblasts, macrophages (clump cells of Koganei), smooth muscle of the sphincter muscle, or posterior epithelium. In another embodiment, the extracellular vesicles as described herein are secreted by the trabecular meshwork cells including trabecular meshwork cells or endothelial
30 cell lining of Schlemm's canal. In another embodiment, the extracellular vesicles as described herein are secreted by cells of the lens including lens epithelium, lens fibers, or lens capsule. In another embodiment, the extracellular vesicles as described herein are secreted by cells of choroid including cuboidal epithelial cells, ependymal cell layer, choroid plexus epithelial cells, or choroidal endothelial cells. In another embodiment, the extracellular vesicles as described
35 herein are secreted by cells of the optic nerve including oligodendrocytes, retinal ganglion

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cell axons, or glial cells. In another embodiment, the extracellular vesicles as described herein are secreted by stem and progenitor cells including mesenchymal stem cells, limbal stem cells, retina stem cells.

[0043] The vitreous and/or aqueous humor extracellular vesicles of the composition as described herein can be any mammalian vitreous or aqueous humor extracellular vesicles. In one embodiment, the composition comprises bovine vitreous and/or aqueous humor extracellular vesicles. In another embodiment, the composition comprises human vitreous and/or aqueous humor extracellular vesicles. In another embodiment, the composition comprises vitreous and/or aqueous humor extracellular vesicles derived from non-human primates, dogs, cats, rodents (*e.g.*, mouse, rat, and guinea pig), horses, cervids, sheep, or pigs.

[0044] As demonstrated herein, the extracellular vesicles of the vitreous and aqueous humor can be isolated, modified to contain one or more exogenous agents, and utilized as a delivery vehicle to delivery the one or more exogenous agents to a target tissue or cell. The exogenous agent can be a therapeutic agent or a diagnostic agent. Suitable therapeutic and diagnostic agents include, without limitation, nucleic acid molecules, proteins and polypeptides, small molecules, hormones, and any combination thereof.

[0045] In one embodiment, the exogenous agent is a therapeutic nucleic acid molecule. The nucleic acid molecule can be single-stranded or double-stranded nucleic acid. Single-stranded nucleic acids include those with phosphodiester, 2'O-methyl, 2' methoxy-ethyl, phosphoramidate, methylphosphonate, and/or phosphorothioate backbone chemistry. In one embodiment, the nucleic acid molecule is a therapeutic nucleic acid molecule selected from a ribonucleic acid molecule (RNA), a deoxyribonucleic acid molecule (DNA), an RNA-DNA hybrid, a modified RNA molecule, modified DNA molecule, or a modified RNA/DNA molecule thereof.

[0046] In one embodiment, the therapeutic nucleic acid molecule is an RNA molecule, such as a small RNA molecule, complementary RNA, a non-coding RNA molecule, siRNA, a pi-RNA molecule, a micro-RNA molecule, a sno-RNA molecule, long non-coding RNA molecule, messenger RNA molecule, ribosomal RNA molecule, an antisense nucleic acid molecule, Locked Nucleic Acid (LNA), antagomir, RNA aptamer, miRNA mimic, miR sponges, CRISPR/Cas gene editing RNA, trans-activating crRNA (tracrRNA), short synthetic RNA composed of a "scaffold" sequence (gRNA), Small Cajal body-specific RNAs (scaRNA), natural cis-antisense siRNAs (cis-nat-siRNAs), trans-acting siRNA (tasiRNA), repeat associated small interfering RNA (rasiRNA), 7SK, transfer-messenger RNA (tmRNA), transfer RNA (tRNA), 7SL RNA, signal recognition particle RNA (SRP), and any combination thereof.

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[0047] In one embodiment, the extracellular vesicles are modified to contain a therapeutic RNA that is suitable for the treatment of an ocular disease or condition. Therapeutic RNAs suitable for the treatment of an ocular disease include, without limitation, siRNA targeting the β 2- adrenoreceptor (SYL040012) for the treatment of glaucoma (Pañeda et al., “Development of SYL040012, a siRNA for treating increased intraocular pressure associated to glaucoma,” AOPT 2013 Scientific Meeting 1:96 (2013), which is hereby incorporated by reference in its entirety), siRNA targeting VEGF (bevasiranib) for the treatment of age related macular degeneration (AMD), siRNA targeting VEGF receptor (siRNA-027) for the treatment of AMD (Kaiser et al., “RNAi-based treatment for neovascular age-related macular degeneration by SiRNA-027,” *Am J Ophthalmol.* 150:33–39 (2010), which is hereby incorporated by reference in its entirety), and siRNA targeting RTP801 (PF-655) for the treatment of AMD and diabetic retinopathy (Nguyen et al., “Phase 1 dose-escalation study of a siRNA targeting the RTP801 gene in age-related macular degeneration patients,” *Eye (Lond)* 26:1099–1105(2012) and Nguyen et al., “Dose-ranging evaluation of intravitreal siRNA PF-04523655 for diabetic macular edema (the DEGAS Study),” *Invest Ophthalmol Vis Sci.* 53:7666–7674 (2012), which are hereby incorporated by reference in their entirety). Other therapeutic RNA molecules suitable for the treatment of ocular diseases that can be introduced to the extracellular vesicles of the composition described herein are described in Guzman-Aranguez et al., “Small-interfering RNAs (siRNAs) as a Promising Tool for Ocular Therapy,” *Br. J. Pharmacol.* 170(4): 730-747 (2013), which is hereby incorporated by reference in its entirety).

[0048] In another embodiment, the isolated extracellular vesicles of the vitreous and/or aqueous humor obtained using the methods described herein, are modified to express or incorporate an mRNA. The mRNA may encode a therapeutic agent that inhibits, down-regulates, reduces a protein expression and/or activity, the excess level of which is associated with an ocular disease, disorder or condition. Such a therapeutic agent may be a peptide, an antibody or other polypeptides or proteins, including any of those described herein. In one embodiment, the mRNA encodes an antibody, a soluble receptor or other binding protein. Typically, a suitable mRNA encodes an antibody that inhibits, down-regulates, or reduces a protein that is present in excess in amount and/or activity in an ocular disease, disorder or condition. In some embodiments, a suitable mRNA encodes an antibody that activates, up-regulates or increases a protein activity that is deficient in an ocular disease, disorder or condition. Exemplary antibodies encoded by mRNAs that can be introduced into the extracellular vesicles of the vitreous and/or aqueous humor as described herein include, but are not limited to, antibodies against VEGF, $\text{TNF}\alpha$, IL-6, ICAM-1, VCAM-1, or soluble receptors such as VEGF receptors (e.g., VEGFR1).

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[0049] Other mRNA molecules that are suitable for the treatment of an ocular disease or condition using the extracellular vesicles as described herein, include for example, and without limitation, mRNA molecules encoding the protein or biologically active fragments of endostatin, angiostatin, tissue inhibitor of metalloproteinase 3 (TIMP3), pigment epithelium derived factor (PEDF), or soluble vascular endothelial growth factor receptor (sFlt-1) for the reduction of neovascularization; mRNA molecules encoding the protein or biologically active fragments of Prph2, Rho, cGMP phosphodiesterase β -subunit (BPDE), Bcl2, PEDF, fibroblast growth factor (FGF-2), ciliary neurotrophic factor (CNTF), and c-mer proto-oncogene tyrosine kinase (Mertk) for the treatment of retinitis pigmentosa; mRNA molecules encoding the protein or biologically active fragments of brain-derived neurotrophic factor (BDNF), CNTF, and GDNF for the treatment of glaucoma; mRNA molecules encoding the protein or biologically active fragments of IL-10 and interleukin-1 receptor agonist (IL-1Ra) for the treatment of uveitis; and mRNA molecules encoding the protein or biologically active fragments of IFN- β and thymidine kinase (TK) for the treatment of retinoblastoma.

[0050] In one embodiment, the extracellular vesicles as described herein are modified to carry one or more of the following mRNA therapeutics, mRNA-1440, mRNA-1851, mRNA MRK-1777, mRNA-1388, mRNA-1325, mRNA-1706, mRNA-1647, mRNA-1653, mRNA-4157, mRNA-2416, mRNA-2905, mRNA AZD-8601, MRG-106, MIR-155, MRG-201, MRG-107, and MRG-110.

[0051] In another embodiment, the mRNA molecule loaded into the extracellular vesicles as described herein encodes a vaccine antigen. The mRNA directs the cells to produce and express the antigenic proteins, either secreted or on the cell surface, much like a native infection would do but without the ability to cause disease or spread. For therapeutic vaccines, using the extracellular vesicles described herein to deliver mRNA-based personalized cancer vaccines to prime the immune system to recognize cancer cells and mount a strong, tailored response to each individual patient's cancer. The extracellular vesicle includes a mRNA that encodes a patient's specific neoantigens, or unique mutations present in that specific patient's tumor.

[0052] In another embodiment, the RNA molecule is catalytic RNA. Ribozymes are catalytic RNAs that function as enzymes and do not require proteins for catalysis. Most known natural ribozymes are self-processing RNAs that catalyze RNA cleavage and ligation reactions. Suitable ribozymes therapeutics that can be delivered using the extracellular vesicles as described herein include, but are not limited to angiozyme, Heptazyme, MY-2, RRz1, OZ1 (RRz1), CCR5 ribozyme, L-TR/Tatneo.

[0053] Other RNA therapeutic molecules that are suitable for the treatment of a disease or condition using the extracellular vesicles as described herein, include for example, and without

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limitation SPC3649 (LNA), Bevasiranib, AGN-745, PF-655, QPI- 1007, TD101, SYL040012, SYL1001, Excellair™, ALN-RSV01, CEQ508, siG12D LODER, TKM-ApoB, TKM-PLK1, ALN-VSP02, ALN-TTR01, Bcr-Abl siRNA, Atu027, I5NP, CALAA-01, FANG vaccine, iPsiRNA, Tat/Rev shRNA, siRNA-EphA2-DOPC, TD101, Atu027, ND-L02-s0201, DCR-PH1, STP705, ALN-GO1, Fitusiran (ALN-AT3SC), ALN-CC5, ALN-AS1, DCR-MYC, TKM 080301, siG12D-LODER, Inclisiran (ALN-PCSSC), PF-655, SYL1001, Bamosiran (SYL040012), QPI-1007, QPI-1002, Patisiran (ALN-TTR02), ISTH0036, EZN-2968 (RO7070179), LerafAON-ETU, AKCEA-APOCIII-LRx, BIIB067 (IONIS-SOD1Rx), AZD5312, Cenersen, IONIS-HTT Rx, IONIS ANGPTL3-LRx, AZD9150, QR-010, SB012, AEG35156, DS-5141b, AKCEA-APO(a)-LRx, Apatorsen (OGX-427), IONIS-HBV Rx, IONIS-GCGR Rx, ASM8, SB010, SB011, G4460, Prexigebersen (BP1001), IONIS-FXI Rx, Aganirsen (GS-101), Eteplirsen (AVI-4658), Alicaforsen, Volanesorsen, IONIS-TTRRx, Custirsen (OGX-011), Lipo-MERIT, IVAC mutanome/warehouse, TNBC-MERIT, CV7201, CV8102, mRNA-1851, mRNA-1440, mRNA MRK-1777, mRNA AZD-8601, mRNA-1325, CV9103, AGS-004, AGS-003-LNG, iHIVARNA-01, AGS-003.

[0054] In one embodiment, isolated extracellular vesicles of the vitreous and/or aqueous humor obtained using the methods described herein, are modified to express or incorporate a nuclease genome editing system useful to edit the genome. Genome editing as described herein may include gene insertions, deletions, modifications (e.g. nucleotide transitions, transversions, insertions or deletions of one or more nucleotides or duplications of any nucleotide sequence), gene activation and gene silencing. As will be appreciated by one of skill in the art, genome editing may be for the purpose of correcting an undesirable gene mutation, introducing a gene mutation, altering a gene sequence (e.g. to improve, enhance or inhibit gene function), inserting a gene sequence (e.g. to activate or inhibit gene expression), and the like. Examples of nuclease genome editing systems include, but are not limited to, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) nuclease system, e.g. including a targeting gRNA and a CRISPR-associated (Cas) gene, such as CRISPR-Cas9, Transcription Activator-Like Effector Nucleases (TALEN) and mito-TALEN, Zinc-Finger Nucleases (ZFN), and other therapeutic nucleic acids, e.g. small interfering RNA, micro RNA, anti-microRNA, antagonist, small hairpin RNA, and aptamers (RNA, DNA or peptide based (including affimers)).

[0055] In one embodiment, the extracellular vesicles of the composition described herein are genetically modified to express or incorporate a CRISPR nuclease system, such as a CRISPR/Cas9 Type II genome editing system, including a Cas nuclease, and a guide RNA (gRNA), which comprises a fusion of trans-activating RNA (tracrRNA) and CRISPR RNA (crRNA). CRISPR RNA includes a targeting RNA sequence and a distinctive array of non-

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coding direct RNA repeats. The crRNA and tracrRNA are related to the selected Cas nuclease. As one of skill in the art will appreciate, the crRNA and tracrRNA (components of the gRNA) and the Cas nuclease are indicated to be "related" which means that the crRNA and tracrRNA are specific for and recognized by one or more particular Cas nucleases.

5 **[0056]** In one embodiment, the CRISPR nuclease system is designed to edit one or more gene defects associated with an ocular condition. For example, and without limitation, the CRISPR nuclease system may be designed to edit the VEGF gene that is overexpressed in age-related macular degeneration as described in Kim et al., "Genome Surgery Using Cas9 Ribonucleoproteins for the Treatment of Age-Related Macular Degeneration," *Genome Research* 10 27:419-426 (2017), which is hereby incorporated by reference in its entirety. In another embodiment, the CRISPR nuclease system may be designed to inactivate the *Nrl* or *NR2e3* genes for the purpose of preventing degeneration associated with retinitis pigmentosa as described by Zhu et al., "Gene and Mutation Independent Therapy via CRISPR-Cas9 Mediated Cellular Reprogramming in Rod Photoreceptors," *Cell Res.* 27:830-833 (2017), which is hereby
15 incorporated by reference in its entirety.

[0057] In another embodiment, the nucleic acid molecule is a DNA molecule. Suitable DNA molecules include, without limitation, a small DNA molecule, a cDNA molecule, an oligonucleotide, a locked Nucleic Acid (LNA), a deoxyribonucleic acid aptamer, a deoxyribonucleic acidzyme (DNAzymes), and any combination thereof.

20 **[0058]** In one embodiment, the therapeutic nucleic acid includes genomic sequences, *e.g.*, cDNA sequences and smaller engineered gene segments that express, or may be adapted to express, proteins, polypeptides, fusion proteins, antibodies, and protein/peptide variants. The nucleic acid may comprise a contiguous nucleic acid sequence of about 5 to about 12000 or more nucleotides, nucleosides, or base pairs. Therapeutic nucleic acid molecules in accordance with
25 this aspect of the invention may encode cytokines, enzymes, hormones, natural agonists and antagonists of proteins involved in disease, etc. Therapeutic nucleic acid molecules also include biologically functional equivalents of a therapeutic nucleic acid proven to benefit in the treatment or prevention of a disease or health-related condition. Accordingly, sequences that have about 70% to about 99% sequence identity to a known nucleic acid molecule are suitable
30 therapeutic nucleic acid molecules in accordance with this aspect of the present invention.

[0059] In one embodiment, the extracellular vesicles are modified to contain a therapeutic DNA molecule that is suitable for the treatment of an ocular disease or condition. Suitable therapeutic DNA molecules include for example, and without limitation, DNA molecules encoding the protein or biologically active fragments of endostatin, angiostatin, tissue inhibitor
35 of metalloproteinase 3 (TIMP3), pigment epithelium derived factor (PEDF), or soluble vascular

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endothelial growth factor receptor (sFlt-1) for the reduction of neovascularization; DNA molecules encoding the protein or biologically active fragments of Prph2, Rho, cGMP phosphodiesterase β -subunit (BPDE), Bcl2, PEDF, fibroblast growth factor (FGF-2), ciliary neurotrophic factor (CNTF), and c-mer proto-oncogene tyrosine kinase (Mertk) for the treatment of retinitis pigmentosa; DNA molecules encoding the protein or biologically active fragments of brain-derived neurotrophic factor (BDNF), CNTF, and GDNF for the treatment of glaucoma; DNA molecules encoding the protein or biologically active fragments of IL-10 and interleukin-1 receptor agonist (IL-1Ra) for the treatment of uveitis; and DNA molecules encoding the protein or biologically active fragments of IFN- β and thymidine kinase (TK) for the treatment of retinoblastoma. Other suitable DNA therapeutics that can be introduced into the extracellular vesicles of the composition as described herein are known in the art, *see* Liu et al., “Gene Therapy for Ocular Diseases,” *Postgrad. Med. J.* 87(1029): 487-95 (2011), which is hereby incorporated by reference in its entirety.

[0060] In another embodiment the therapeutic DNA molecule suitable for treatment of an ocular disease which is loaded into the extracellular vesicles of the composition described herein is an aptamer. Suitable aptamers include, for example and without limitation, Macugen/pegaptanib (NX1838) targeting the activity of VEGF for the treatment of ocular neovascular diseases, Fovista/pegpleranib (NX1975) targeting the activity of PDGF B-chain for the treatment of age-related macular degeneration, and Zimura/ARC1905 targeting the activity of complement component 5 (C5) for the treatment of age-related macular degeneration (*see* Drolet et al., “Fit for the Eye: Aptamers in Ocular Disorders,” *Nucleic Acid Ther.* 26(3):127-146 (2016), which is hereby incorporated by reference in its entirety). Other suitable aptamers include RNA aptamer (RB006 or pegnivacogin), ARC19499(BAX499), REG1 (RB006 & RB007), ARC1905, TAR decoy, RRE decoy.

[0061] In one embodiment, a combination of therapeutic RNA and DNA molecules are introduced into the extracellular vesicles of the composition described herein. In one embodiment, the combination of therapeutic RNA and DNA molecules work in concert for the treatment of an ocular disease. For example, and without limitation, siRNA molecules capable of silencing the expression of mutant rhodopsin expression can be administered in combination with a DNA molecule encoding the wildtype rhodopsin gene for the treatment of retinitis pigmentosa (O'Reilly et al., “RNA interference-mediated suppression and replacement of human rhodopsin *in vivo*,” *Am J Hum Genet.* 81:127–135 (2007), which is hereby incorporated by reference in its entirety).

[0062] In another embodiment, the nucleic acid is a diagnostic nucleic acid. A diagnostic nucleic acid is a nucleic acid that can be applied in the diagnosis of a disease or health-related

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condition. A diagnostic nucleic acid sequence that encodes one or more reporter proteins. A “reporter protein” refers to an amino acid sequence that, when present in a cell or tissue, is detectable and distinguishable from other genetic sequences or encoded polypeptides present in cells. In some embodiments, a therapeutic nucleic acid molecule may be fused to the diagnostic nucleic acid encoding a reporter protein. For example, the two nucleic acid molecules may be linked to the same promoter by, for example, an internal ribosome entry site, or a bi-directional promoter. Using such techniques, expression of the therapeutic nucleic acid and diagnostic nucleic acid correlate. Thus, when the composition is used in the methods as described herein, one may gauge the location, amount, and duration of expression of a therapeutic nucleic acid.

[0063] Preferably, a reporter sequence encodes a protein that is readily detectable either by its presence, its association with a detectable moiety, or by its activity that results in the generation of a detectable signal. In certain aspects, a detectable moiety may include a radionuclide, a fluorophore, a luminophore, a microparticle, a microsphere, an enzyme, an enzyme substrate, a polypeptide, a polynucleotide, a nanoparticle, and/or a nanosphere, all of which may be coupled to an antibody or a ligand that recognizes and/or interacts with a reporter. Exemplary diagnostic nucleic acid molecules include, without limitation, nucleic acid molecules encoding β -lactamase, β -galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, G-protein coupled receptors (GPCRs), somatostatin receptors, CD2, CD4, CD8, the influenza hemagglutinin protein, symporters (such as NIS) and others well known in the art.

[0064] In one embodiment, the extracellular vesicles of the composition described herein are modified to include a naked nucleic acid molecule, e.g. naked DNA or naked RNA. In another embodiment, the nucleic acid is packaged in an expression vector suitable for expression in prokaryotes or eukaryotes or both, preferably for expression in mammalian cells. Suitable expression vectors include viral vectors (*e.g.*, adenoviral vector, adeno-associated viral vector, lentiviral vector, vaccinia viral vector, retroviral vector, herpes viral vector), bacterial vectors, plasmid vectors, artificial chromosomes, bacteriophages, or any combination thereof. Expression vectors generally contain regulatory sequences and other necessary elements for the translation and/or transcription of the inserted coding sequence. For example, the coding sequence is preferably operably linked to a promoter and/or enhancer to help control the expression of the desired gene product. Promoters used in biotechnology are of different types according to the intended type of control of gene expression. They can be generally divided into constitutive promoters, tissue-specific or development- stage-specific promoters, inducible promoters, and synthetic promoters. Depending on the vector system and host utilized, any

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number of suitable transcription and translation elements may be used. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferably used.

[0065] Methods of constructing expression vectors containing the desired nucleic acid molecules and appropriate transcriptional and translational control elements are well known in the art. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described in Sambrook et al., Molecular Cloning, A Laboratory Manual (Cold Spring Harbor Press, Plainview, N.Y., 1989), and Ausubel et al, Current Protocols in Molecular Biology (John Wiley & Sons, New York, N.Y., 1989), which are hereby incorporated by reference in their entirety.

[0066] The extracellular vesicles can be loaded with the nucleic acid or nucleic acids of interest using techniques known in the art, such as, for example, electroporation. Electroporation involves introducing pores into the vesicles using a pulse of electricity (e.g., 100-400 V/cm), where the nucleic acid(s) enter the vesicles through the pores. The extracellular vesicles can alternatively be loaded with nucleic acid(s) of interest using microinjection or particle bombardment. Alternatively, the extracellular vesicles can be loaded using lipofection or transfection using commercially available kits and reagent, or by transformation using heat shock.

[0067] In another embodiment, the vitreous and/or aqueous humor vesicles are loaded with a therapeutic protein and/or peptide for delivery. In one embodiment, the therapeutic protein is an exogenous protein or peptide. Exogenous refers to a protein or peptide with which the vesicle is not normally associated.

[0068] The protein and/or peptide to be loaded into the vesicles is chosen based the desired effect of that protein and/or peptide on the target cell. A single protein or peptide may be incorporated into the vesicles. Alternatively, more than one protein and/or peptide may be incorporated into the vesicles. The more than one protein and/or peptide may act on the same or different targets to bring about the desired therapeutic and/or preventative effect.

[0069] In one embodiment, the protein and/or peptide to be loaded into the vesicles is an antibody or antibody fragment. The term "antibody" as referred to herein includes whole antibodies (*i.e.*, two heavy chains and two light chains), antibody binding fragments thereof, *e.g.*, single chain antibodies (scFv), single domain antibodies (*e.g.*, nanobodies or Fv), Fab, Fab', F(ab')₂, and, variants thereof, *e.g.*, tandem scFv, Fd fragments, diabodies, triabodies. These antibody fragments may be obtained using conventional techniques known to those of skill in the art, and the fragments may be screened for utility in the same manner as intact antibodies

[0070] Antibody and antibody fragments disclosed herein can be mono-valent, bi-valent, or tri-valent with regard to binding domains, and the binding domains may be mono-specific, bi-

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specific, or tri-specific in binding specificity by design. Suitable antibodies include monoclonal antibodies or a polyclonal antibody mixture. The antibody may be a chimeric antibody, a CDR-grafted antibody, a humanized antibody or an antigen binding portion of any of the foregoing thereof. Therapeutic antibodies may be derived from a variety of species, including, without
 5 limitation, mouse, human, camel, llama, goat, rabbit, bovine, and cartilaginous fish.

[0071] In one embodiment, the antibody or antigen binding fragment thereof is one that is suitable for the treatment of an ocular disease or condition. Suitable antibodies or antigen binding fragments thereof include, without limitation, those that bind to and preferentially block or reduce the activity of integrins associated with disease, such as an anti- $\alpha_v\beta_3$ integrin antibody and an anti- $\alpha_4\beta_1$ integrin antibody. Other suitable antibodies that can be introduced into the extracellular vesicles of the compositions described herein include, for example and without limitation, an anti-epidermal growth factor receptor antibody, anti-vascular endothelial growth factor (VEGF) receptor antibody, anti-VEGF antibodies, *e.g.*, bevacizumab, ranibizumab, anti-TNF α antibodies, *e.g.*, infliximab and adalimumab, an anti-fibroblast growth factor antibody, an
 10 anti-epidermal growth factor antibody, an anti-CD20 antibody, an anti-CD52 antibody, an anti-CD11a antibody, and anti-IL-2 antibody.

[0072] Other suitable antibodies that can be introduced into the extracellular vesicles of the compositions described herein include, for example and without limitation, abciximab (Reopro), adalimumab (Humira, Amjevita), alefacept (Amevive), alemtuzumab (Campath), basiliximab
 20 (Simulect), belimumab (Benlysta), bezlotoxumab (Zinplava), canakinumab (Ilaris), certolizumab pegol (Cimzia), cetuximab (Erbix), daclizumab (Zenapax, Zinbryta), denosumab (Prolia, Xgeva), efalizumab (Raptiva), golimumab (Simponi, Simponi Aria), inflectra (Remicade), ipilimumab (Yervoy), ixekizumab (Taltz), natalizumab (Tysabri), nivolumab (Opdivo), olaratumab (Lartruvo), omalizumab (Xolair), palivizumab (Synagis), panitumumab (Vectibix),
 25 pembrolizumab (Keytruda), rituximab (Rituxan), tocilizumab (Actemra), trastuzumab (Herceptin), secukinumab (Cosentyx), ustekinumab (Stelara). Additional anti-angiogenesis protein/peptide therapeutics include, without limitation, ramucirumab, axitinib, axitinib, MGCD516, cediranib, olaparib, lestaurtinib, olaparib, cediranib, pazopanib, docetaxel, pazopanib hydrochloride, TRC105, pazopanib, X4p-001, nivolumab, eribulin mesylate,
 30 ketoconazole, therapeutic hydrocortisone, antibody J591, docetaxel, plinabulin, SF1126, carfilzomib, hydroxychloroquine, aldesleukin, bevacizumab, erlotinib, sorafenib, vandetanib, durvalumab, olaparib, cediranib, sapanisertib, ziv-aflibercept, bevacizumab, LY2157299 monohydrate (LY2157299), temozolomide, SGT-53, cediranib maleate, olaparib, bevacizumabosimertinib, regorafenib, itraconazole.

[0073] In another embodiment, the therapeutic protein is an antibody mimetic.

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An “antibody mimetic” encompasses any organic compound, *e.g.*, a peptide or polypeptide, that can specifically bind an antigen like an antibody and is about 3-20kDa. In one embodiment, the antibody mimetic comprises a scaffold which binds its target antigen via amino acids in exposed loops similar to the CDR loops of an antibody. These antibody mimetics include, without
5 limitation, adnectins, lipocalins, Kunitz domain-based binders, avimers, knottins, fynomers, atrimers, and cytotoxic T-lymphocyte associated protein-4 (CTLA4)-based binders (reviewed in Weidle et al., “The Emerging Role of New Protein Scaffold-based Agents for the Treatment of Cancer,” *Cancer Genomics & Proteomics* 10:155-168 (2013), which is hereby incorporated by reference in its entirety).

10 **[0074]** In another embodiment, the therapeutic protein is a protein or peptide inhibitor. Protein or peptide inhibitors can be full-length proteins or biologically active peptide fragments thereof which naturally antagonize or inhibit the action or activity of one or more receptors, enzymes, hormones, proteases, kinases, growth factors, signal transduction pathways, transcription factors, etc. that are associated with a disease or condition to be treated. The
15 protein or peptide inhibitor may act as a dominant negative receptor or ligand, or a decoy receptor or ligand.

[0075] In one embodiment, the extracellular vesicles of the composition as described herein are modified to contain a protein or peptide inhibitor that is suitable for the treatment of an ocular disease. For example, the protein or peptide inhibitor may be an inhibitor of
20 angiogenesis. Suitable protein/peptide inhibitors of angiogenesis include, without limitation, angiostatin, including full-length angiostatin and biologically active fragments and analogs thereof, and endostatin, including full-length endostatin and biologically active fragments and analogs thereof; other collagen derived peptides, such as tumstatin peptide, tumstatin fragment, and pentastatin; RGD containing peptides, such as Cilengitide, and other fibronectin derived
25 peptides; and peptides derived from laminin, such as C16Y and C16S (*see* Rosca et al., “Anti-angiogenic Peptides for Cancer Therapeutics,” *Curr. Pharm. Biotechnol.* 12(8): 1101-1116 (2011), which is hereby incorporated by reference in its entirety).

[0076] Other suitable protein or peptide inhibitors that can be loaded into the extracellular vesicles include, without limitation, integrin antagonists, *e.g.*, LFA-1, VLA-4, Mac-1, ICAM-1,
30 ICAM-2, VCAM antagonists, chemokine antagonists, *e.g.*, MCP-1, MCP-5, MCP-3, MIP1 α , CCR5, RANTES antagonists, and selectin antagonists, *e.g.*, E-selectin, P-selectin, and L-selectin antagonists.

[0077] Other suitable protein or peptide inhibitors that can be loaded into the extracellular vesicles include, without limitation, anti-VEGF agents, Ranibizumab (Lucentis, Genentech,
35 South San Francisco), aflibercept (Eylea, Regeneron Pharmaceuticals, Tarrytown, N.Y.),

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Lucentis, Bevacizumab (Avastin, Genentech), Corticosteroids, Intravitreal steroids, sustained-release biodegradable dexamethasone implant, Ozurdex (Allergan, Irvine, Calif.), Vitreolytics including Ocriplasmin (Jetrea, ThromboGenics, Leuven, Belgium), anti-PDGF therapies, RTH258, a small, humanized anti-VEGF antibody fragment that inhibits all isoforms of VEGF-A, anti-VEGF DARPIn (abicipar pegol), the anti-PDGF agent Fovista (Ophthotech, New York), and Iluvien, a non-biodegradable implant indicated for DME that elutes the steroid fluocinolone acetonide.

[0078] The exogenous protein and/or peptide can be introduced into the vesicles by a number of different techniques. In one embodiment, the vesicles are loaded by electroporation or the use of a transfection reagent. Electroporation conditions may vary depending on the charge and size of the therapeutic cargo. Typical voltages are in the range of 20 V/cm to 1000 V/cm, such as 20 V/cm to 100 V/cm with capacitance typically between 25 μ T and 250 μ T, such as between 25 μ T and 125 μ T. A voltage in the range of 150 mV to 250 mV, particularly a voltage of 200 mV is preferred for loading vesicles with an antibody.

[0079] Alternatively, the vesicles may be loaded with exogenous protein and/or peptide using a transfection reagent. Despite the small size of the vesicles, conventional transfection agents may be used for transfection of vesicles with protein and/or peptide. Preferred transfection reagents for use in accordance with the present invention include cationic liposomes.

[0080] In another embodiment, extracellular vesicles may also be loaded by transforming or transfecting a host cell with a nucleic acid construct which expresses therapeutic protein or peptide of interest, such that the therapeutic protein or peptide is taken up into the extracellular vesicles as the vesicles are produced from the cell.

[0081] In another embodiment, the vitreous and/or aqueous humor vesicles are loaded with a therapeutic small molecule for delivery. In one embodiment, the small molecule is a small molecule used to treat ocular disease. Suitable ophthalmic therapeutic agents that can be loaded into the vitreous and/or aqueous humor extracellular vesicles included, without limitation, a carbonic anhydrase inhibitor, *e.g.*, brinzolamide; a β adrenergic blocker, such as betaxolol, carteolol, levobunolol, metipranolol, timolol maleate, and timolol hemihydrate; an α 2 adrenergic agonists, such as Apraclonidine, Lopindine, Brimonidine, and Alphagan; a prostaglandin, such as bimatoprost, loteprednol, and bromfenac; anti-infective agents such as antibiotics, anti-fungal agents, and anti-viral agents; T-cell immune suppression agents like cyclosporine, lipophilic steroids, and antibiotic and steroid combinations; small molecule tyrosine kinase inhibitors (TKI) such as sunitinib and sorafenib.

[0082] Additional therapeutics that can be loaded into the extracellular vitreous and/or aqueous humor vesicles for delivery to ocular tissues as described herein include, without

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limitation, Iquix (generic name: levofloxacin), natacyn (generic name: natamycin), tobrex
 (generic name: tobramycin), polytrim (generic name: polymyxin b/trimethoprim), ciloxan
 (generic name: ciprofloxacin), viroptic (generic name: trifluridine), moxeza (generic name:
 5 oxifloxacin), zymar (generic name: gatifloxacin), besivance (generic name: besifloxacin),
 vigamox (generic name: moxifloxacin), zirgan (generic name: ganciclovir), azasite (generic
 name: azithromycin), ak-chlor (generic name: chloramphenicol), ak-poly-bac (generic name:
 bacitracin/polymyxin b), ak-tob (generic name: tobramycin), betadine ophthalmic solution
 (generic name: povidone iodine), bleph-10 (generic name: sulfacetamide sodium), chloromycetin
 ophthalmic (generic name: chloramphenicol), chloroptic (generic name: chloramphenicol),
 10 dendrid (generic name: idoxuridine), eyemycin (generic name: erythromycin), garamycin
 ophthalmic (generic name: gentamicin), genoptic (generic name: gentamicin), gentacidin
 (generic name: gentamicin), gentak (generic name: gentamicin), gentasol (generic name:
 gentamicin), ilotycin (generic name: erythromycin), isopto cetamide (generic name:
 sulfacetamide sodium), neo-polycin (generic name: bacitracin/neomycin/polymyxin b), neocidin
 15 (generic name: acitracin/neomycin/polymyxin b), neocidin ophthalmic solution (generic name:
 gramicidin/neomycin/polymyxin b), neosporin ophthalmic (generic name:
 gramicidin/neomycin/polymyxin b), ocu-chlor (generic name: chloramphenicol), ocu-mycin
 (generic name: gentamicin), ocu-spore-b (generic name: bacitracin/neomycin/polymyxin), ocu-
 spore-g (generic name: gramicidin/neomycin/polymyxin b), ocu-tracin (generic name:
 20 bacitracin), ocuflox (generic name: ofloxacin), polycin-b (generic name: bacitracin/polymyxin
 b), quixin (generic name: levofloxacin), roymicin (generic name: erythromycin), sulf-10 (generic
 name: sulfacetamide sodium), terramycin with polymyxin b sulfate (generic name:
 oxytetracycline/polymyxin b), tobrasol (generic name: tobramycin), tomycine (generic name:
 tobramycin), vira-a (generic name: vidarabine), vitrasert (generic name: ganciclovir), zymaxin,
 25 atropine, azopt, bacitracin, betadine, betaxolol, betoptic, brinzolamide, bss (balanced salt
 solution), carbachol, cefazolin, celluvisc, chloramphenicol, ciloxan, ciprofloxacin, cosopt,
 demecarium, dexamethasone, dipivefrin, dorzolamide, epinephrine, fluorescein, flurbiprofen,
 physostimine, gentamicin, pilocarpine, goniosol, polymyxin b, gramicidin, prednisolone,
 humorsol, proparacaine, hylartin, propine, hypertonic nacl, puralube, indocyanine green, rose
 30 bengal, itraconazole, sodium hyaluronate, latanoprost, suprofen, mannitol, terramycin,
 methazolamide, timolol, miconazole, tobramycin, miostat, triamcinolone, muro 128, trifluridine,
 neomycin, tropicamide, neptazane trusopt, ocuflox, vidarabine, ofloxacin, vira-a,
 oxytetracycline, viroptic, phenylephrine, xalatan, NVC-422, FST-100, Luveniq, ESBA105,
 Mapracorat (ZK 245186/BOL-303242-X), Nepafenac 0.3%, DexaSite (or ISV-305), AzaSite
 35 Plus (or ISV-502), CF101, and Lifitegrast (SAR 1118).

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[0083] Additional therapeutics that can be loaded into the extracellular vitreous and/or aqueous humor vesicles for delivery to ocular tissues for the treatment of glaucoma as described herein include, without limitation, prostaglandin analogs include xalatan® (latanoprost), lumigan® (bimatoprost), travatan z® (travoprost), and zioptan™ (tafluprost), beta blockers such as timolol, alpha agonists [alphagan®p (brimonidine), iopidine®], carbonic anhydrase inhibitors including [trusopt® (dorzolamide), azopt® (brinzolamide)] as well as diamox (acetazolamide) and neptazane® (methazolamide) and brinzolamide, combined medications including cosopt®, and also as a preservative-free formulation (cosopt® pf), combigan, simbrinza®, iopidine®, apraclonidine hydrochloride 0.5%, 1%, alphagan® , brimonidine tartrate 0.1%, 0.15%, timolol maleate usp, timolol maleate 0.5%, betoptic® , betaxolol hydrochloride 0.25%, 0.5%, betagan®, levobunolol hydrochloride ophthalmic solution, usp 0.25%, 0.5%, optipranolol®, metipranolol 0.3% istalol® timolol maleate ophthalmic solution 0.5%, timoptic-xe®, timolol maleate ophthalmic gel forming solution 0.25%, 0.5%, betimol®, timolol hemihydrate 0.25%, 0.5%, azopt®, brinzolamide ophthalmic suspension 1%, neptazane®, methazolamide, trusopt®, dorzolamide hydrochloride 2%, diamox® sequels®, acetazolamide, isopto® carpine, pilocarpine hydrochloride 1%, 2%, 4%, isopto® carbachol, 0.75%, 1.5%, 3%, pilopine HS® hydrochloride gel 4%, pilocarpine hydrochloride ophthalmic solution usp, pilocarpine hydrochloride 1%, 2%, 4%, combigan™, brimonidine tartrate & timolol maleate, cosopt®, dorzolamide hydrochloride & timolol maleate, simbrinza® suspension, brinzolamide/brimonidine tartrate ophthalmic suspension 1%/0.2%, Travatan z®, travaprost 0.004%, lumigan®, bimatoprost 0.01%, 0.03%, zioptan™, tafluprost ophthalmic solution 0.0015%, xalatan®, latanoprost 0.005%, ROCK inhibitor, Y-27632, ATS907, ATS8535, AR-12286, AR-13324, AMA0076, BOL-303259-X.

[0084] Additional therapeutics that can be loaded into the extracellular vitreous and/or aqueous humor vesicles for delivery to ocular tissues for the treatment of dry eye include, without limitation, restasis ophthalmic, lacrisert ophthalmic, systane ultra ophthalmic, carboxymethylcellulose sodium ophthalmic, soothe xp ophthalmic, systane (propylene glycol) ophthalmic, freshkote ophthalmic, refresh optive advanced ophthalmic, genteal gel ophthalmic, retaine MGD (pf) ophthalmic, clear eyes itchy eye relief, systane balance ophthalmic, refresh tears, refresh liquigel ophthalmic, hypotears, clear eyes redness relief, bion tears (pf), peg 400-propylene glycol ophthalmic, refresh optive sensitive (pf) ophthalmicm, refresh plus ophthalmic, tears naturale free (pf), liquitears, cyclosporine ophthalmic, genteal pm ophthalmic, systane nighttime ophthalmic, genteal severe ophthalmic, systane gel ophthalmic, refresh lacri-lube ophthalmic, refresh p.m. ophthalmic, eye drops, isopto tears, puralube ophthalmic, theratears, polyvinyl alcohol ophthalmic, polyethylene glycol-polyvinyl alcohol eye, tears naturale pm, tears naturale forte, dextran 70-hypromellose ophthalmic, lubrifresh pm ophthalmic, lubricant eye

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drops, refresh celluvisc ophthalmic, carboxymethylcellulose-glycerin(pf) ophthalmic, lubricant
 eye (pg-peg 400), systane liquid gel ophthalmic, soothe hydration ophthalmic, refresh classic (pf)
 ophthalmic, refresh optive ophthalmic, systane ultra (pf) ophthalmic, systane (pf) ophthalmic,
 soothe lubricant ophthalmic, clear eyes complete ophthalmic, retaine pm ophthalmic, eye drops
 5 (with povidone), artificial tears (polyvinyl alcohol), visine totality ophthalmic, opti-clear
 ophthalmic, tetrahydrozoline-peg ophthalmic, moisture drops ophthalmic, tears again, tears pure,
 goniosoft ophthalmic, gonak ophthalmic, lubricating drops ophthalmic, polyvinyl alcohol-
 povidone ophthalmic, white petrolatum-mineral oil ophthalmic, artificial tears (hypromellose),
 genteal mild ophthalmic, goniotaire ophthalmic, tearfair for the eye, nature's tears, sterile eye
 10 drops, ultra fresh ophthalmic, ultra fresh pm ophthalmic, peg 400-hypromellose-glycerin
 ophthalmic, naphazoline-peg 300 ophthalmic, naphazoline-hypromellose ophthalmic, propylene
 glycol ophthalmic, all clear AR ophthalmic, propylene glycol-glycerin ophthalmic, pure and
 gentle eye, for sty relief ophthalmic, artificial tears (dextran-hypromellose-glycerin), sterile
 lubricant ophthalmic, hydroxypropyl cellulose ophthalmic, eye drops advanced relief, lubricant
 15 eye, light mineral oil-mineral oil ophthalmic, dry eye relief, redness relief ophthalmic,
 tetrahydralazine-dextran70-peg400-povdn ophthalmic, artificial tears with lanolin, eye lubricant
 combination no.1, carboxymethylcellulose sod-hypromell ophthalmic, advanced eye relief,
 naphazoline-glycerin ophthalmic, genteal mild to moderate ophthalmic, artificial tears
 (hypromellose) (pf), carboxymethylcellulose-glycerin ophthalmic, lubricant redness reliever
 20 ophthalmic, artificial tears (glycerin/propylene glycol), naphazoline-zinc sulfate-glycerin
 ophthalmic, lubricant eye drops (glycerin-propylene glycol), redness reliever lubricant
 ophthalmic, goniovisc ophthalmic, advanced eye relief (mo-wpet) ophthalmic, refresh contacts
 ophthalmic, dextran 70-hypromellose (pf) ophthalmic, artificial tears (pf), natural tears (pf),
 tetrahydrozoline-peg 400-hyprom-glyc ophthalmic, lubricant eye (dextran 70/hypromellose),
 25 artificial tears (petrolatum/mineral oil), eye drop tears, povidone ophthalmic, peg 400-propylene
 glycol (pf) ophthalmic, polyvinyl alcohol-povidone (pf) ophthalmic, advanced formula eye
 drops, retaine cmc ophthalmic, light mineral oil-mineral oil (pf) ophthalmic,
 carboxymethylcellulose-glycerin-polysorb 80 ophthalmic, maximum redness relief ophthalmic,
 lubricant dry eye relief, eq gentle ophthalmic, carboxymethyl-glycerin-polysorb 80-pf
 30 ophthalmic, ultra lubricant eye, moisturizing lubricant ophthalmic, lubricating plus ophthalmic,
 revive plus ophthalmic, naphazo hcl-hyprome-ps 80-zn sulf ophthalmic, akwa tears (polyvinyl
 alcohol) ophthalmic, visine tears, visine tired eye relief, visine max redness relief, visine
 advanced redness relief, refresh optive advanced (pf) ophthalmic, tetrahydrozoline-zinc-peg 400-
 hypromello-glycerin ophthalmic, retaine hpmc ophthalmic, lubricant eye (propylene glycol)
 35 ophthalmic, lubricant eye (carboxymethylcellulose-glycerin) ophthalmic, lubricant eye (cmc-

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glycerin) (pf) ophthalmic, lubricant eye (pg-peg 400) (pf) ophthalmic, lubricating relief ophthalmic, lubricant gel ophthalmic, restore tears ophthalmic, lubricant plus ophthalmic, natural balance tears ophthalmic, clear eyes cooling comfort ophthalmic, clear eyes maximum redness relief ophthalmic, genteal tears ophthalmic, tears again (pva) ophthalmic, artificial tears (dextran
 5 70-hypromellose) ophthalmic, artificial tears (polyvinyl alcohol/povidone) ophthalmic, artificial tears (pg400-hypromellose-glycerin) ophthalmic, genteal tears (dxtrn-hpm-gly) ophthalmic and Xiidra[®] (lifitegrast ophthalmic solution) 5% or any other percentage or combination.

[0085] In one embodiment, the vitreous and/or aqueous humor extracellular vesicles of the composition are further modified to express or display a eukaryotic cell-specific targeting
 10 molecule or moiety on the outer surface of the vesicular body. In one embodiment, the targeting moiety is a peptide which is expressed as a fusion protein with a transmembrane protein typically expressed on the surface of the extracellular vesicle. Suitable peptides are those which bind to cell surface moieties such as receptors or their ligands found on the cell surface of the cell to be targeted. Examples of suitable targeting moieties are short peptides (typically less than 100
 15 amino acids in length, for example less than 50 amino acids in length, less than 30 amino acids in length, to a minimum length of 10, 5, 3, 2, or 1 amino acid(s)), full-length proteins, antibodies or antigen binding fragments and derivatives thereof (e.g., Fab, Fab', F(ab')₂, scFv, Fv, etc.), and complete proteins, so long as the targeting moiety can be expressed on the surface of the extracellular vesicle and does not interfere with insertion of the membrane protein into
 20 the extracellular vesicle. Typically the targeting peptide is heterologous to the transmembrane extracellular vesicle protein.

[0086] Targeting moieties can be selected to target the extracellular vesicle to a particular tissue type such as, for example, ocular, muscle, brain, liver, pancreas, lung, etc., or to target a diseased tissue such as a tumour. In a one embodiment of the present invention, the extracellular
 25 vesicles are targeted to ocular tissue.

[0087] In one embodiment, extracellular vesicles can be targeted to ocular tissues by expressing moieties or ligands recognized by ocular tissue influx transporters on the outer body surface of the extracellular vesicles. Several amino acid and peptide transporters are expressed on ocular tissue and cells. For example, the amino acid transporter ASCT1 (SLC1A4) is
 30 expressed in the cornea and primary corneal epithelial cells, and the amino acid transporter ASCT2 (SLC1A5) is expressed on retinal Muller cells. B^{0,+} (SLC6A14) is a neutral and cationic amino acid transporter with broad substrate specificity expressed in corneal epithelium. Lat1 (SLC7A5) is expressed in human cornea, and LAT2 (SLC7A8) is expressed in retinal pigment epithelial cells. The peptide transporters, PEPT1 and PEPT2 are expressed in corneal epithelium
 35 and retinal Muller cells. Other than amino acid and peptide transporters, organic cation/anion

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(SLC22), monocarboxylate (SLC16), and nucleoside transporters (SLC 28 and 29) have also been identified on various ocular tissues. Accordingly, extracellular vesicles can be decorated with transporter-specific targeting moieties to direct delivery of the therapeutic cargo carried by the extracellular vesicle. Suitable targeting moieties include, without limitation, L-aspartate, gamma-glutamate, and phenylalanine to direct delivery via the B^(0,+) amino acid transporter (*see e.g.*, Majumdar et al., “Transcorneal Permeation of L- and D-aspartate Ester Prodrugs of Acyclovir: Delineation of Passive Diffusion Versus Transporter involvement,” *Pharm Res.* 26(5):1261–9 (2009), Anand et al., “Amino Acid Prodrugs of Acyclovir as Possible Antiviral Agents against Ocular HSV-1 Infections: Interactions with the Neutral and Cationic Amino Acid Transporter on the Corneal Epithelium,” *Curr Eye Res.* 29(2–3):153–66 (2004), and Dun et al., “Functional and Molecular Analysis of D-serine Transport in Retinal Muller Cells,” *Exp Eye Res.* 84(1):191–9 (2007), which are hereby incorporated by reference in their entirety); L-valine, Glycine-Valine, Valine-Valine, Tyrosine-Valine moieties to target delivery via oligopeptide transporters on the retina and cornea (*see e.g.*, Anand and Mitra, “Mechanism of Corneal Permeation of L-valyl Ester of Acyclovir: Targeting the Oligopeptide Transporter on the Rabbit Cornea,” *Pharm Res.* 19(8):1194–202 (2002), Gunda et al., “Corneal Absorption and Anterior Chamber Pharmacokinetics of Dipeptide Monoester Prodrugs of Ganciclovir (GCV): In vivo Comparative Evaluation of these Prodrugs with Val-GCV and GCV in Rabbits,” *J Ocul Pharmacol Ther.* 22(6):465–76 (2006), Majumdar et al. “Dipeptide Monoester Ganciclovir Prodrugs for Treating HSV-1-induced Corneal Epithelial and Stromal Keratitis: In vitro and In vivo Evaluations,” *J Ocul Pharmacol Ther.* 21(6):463–74 (2005), Katragadda et al., “Modulation of P-glycoprotein-mediated Efflux by Prodrug Derivatization: an Approach Involving Peptide Transporter-mediated Influx across Rabbit Cornea,” *J Ocul Pharmacol Ther.* 22(2):110–20 (2006), Kansara et al., “Dipeptide Monoester Ganciclovir Prodrugs for Transscleral Drug Delivery: Targeting the Oligopeptide Transporter on Rabbit Retina,” *J Ocul Pharmacol Ther.* 23(4):321–34 (2007), which are hereby incorporated by reference in their entirety), biotin to target delivery via the sodium-dependent multiple vitamin transporter on the retina (*see e.g.*, Janoria et al., “Vitreous Pharmacokinetics of Biotinylated Ganciclovir: Role of Sodium-dependent Multivitamin Transporter Expressed on Retina,” *J Ocul Pharmacol Ther.* 25(1):39–49 (2009), which is hereby incorporated by reference in its entirety) and glucose to target delivery to GLUT1 receptor on retinal pigment epithelial cells (*see e.g.*, Dalpiaz et al., “Molecular Mechanism Involved in the Transport of a Prodrug Dopamine Glycosyl Conjugate,” *Int J Pharm.* 336(1):133–9 (2007), which is hereby incorporated by reference in its entirety).

[0088] The peptide targeting moiety is expressed on the surface of the extracellular vesicles by expressing it as a fusion protein with an extracellular vesicle transmembrane protein.

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A number of proteins are known to be associated with extracellular vesicles; that is they are incorporated into the extracellular vesicle as it is formed. The preferred proteins for use in targeting the extracellular vesicles of the present invention are those which are transmembrane proteins. Examples include but are not limited to Lamp-1, flotillin, Syntaxin-3, CD9, CD63, CD81, HLA-DM (MHC II), immunoglobulins, MHC-I or MHC-II components, and tetraspanins.

[0089] In other embodiments, a specific targeting moiety does not need to be included in the extracellular vesicle. For example, extracellular vesicles may be administered directly to the site where therapy is required. Alternatively, delivery by, for example, periocular or intraocular administration may be sufficient to generate the desired response.

[0090] In some embodiments, particularly, where the extracellular vesicles of the composition are modified to contain an exogenous therapeutic agent, the composition further comprises a pharmaceutically acceptable carrier. A "pharmaceutically acceptable carrier" (excipient) is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering the extracellular vesicles of the composition to a subject. Typical pharmaceutically acceptable carriers include, but are not limited to, binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc); fillers (*e.g.*, lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc); lubricants (*e.g.*, magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc); disintegrates (*e.g.*, starch, sodium starch glycolate, etc); or wetting agents (*e.g.*, sodium lauryl sulphate, etc).

[0091] The compositions provided herein may additionally contain other adjunct components conventionally found in pharmaceutical compositions. Thus, for example, the compositions may contain additional compatible pharmaceutically-active materials or may contain additional materials useful in physically formulating various dosage forms of the composition of present invention, such as dyes, flavouring agents, preservatives, antioxidants, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions provided herein.

[0092] In one embodiment, the composition of vitreous and/or aqueous humor extracellular vesicles is formulated in a slow or sustained release material. For example, in one embodiment, the composition can be formulated to include a thin film coating that slowly releases the extracellular vesicles of the composition to the target area or target tissue. The methods and materials that can be used to prepare coatings suitable for slow or sustained release coatings are well known to those skilled in the art. Suitable coatings should be both biocompatible

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and compatible with the extracellular vesicular composition. In one embodiment, the thin film is composed of bioabsorbable polymer(s). Examples of suitable bioabsorbable elastomers are described in U.S. Pat. Nos. 5,468,253 to Bezwada and 6,627,246 to Mehta. Useful polymers include mixtures of L-lactide, D-lactide, epsilon-caprolactone, and glycolide. The relative composition of these mixtures can be used to control the rate of coating hydrolysis and adsorption, the rate of extracellular vesicle release, and the strength of the film. Other polymeric materials that can be used to prepare thin films suitable for slow release include (but are not limited to) polyamides, polyalkylenes oxalates, poly(amino acids), copoly(ether-esters), poly(iminocarbonates), polyorthoesters, poly(anhydrides), and blends thereof. Naturally occurring polymers that can be degraded in the eye for slow release ocular compositions include hyaluronic acid, absorbable biocompatible polysaccharides such as chitosan or starch, fibrin, elastin, fibrinogen, collagen, and fatty acids (and esters thereof). In one embodiment, polymers containing the composition of extracellular vesicles can be applied to, for example, an implant, by spraying solutions containing dissolved polymer containing the composition of extracellular vesicles, to the surface to be coated or by dipping a portion of the implant in these solutions. Thin films typically provide sustained delivery for a few weeks until the therapeutic in the film is exhausted. The thickness will depend on how long delivery is desired and the concentration of extracellular vesicle loading. Typically, the thickness is 5-30 microns or less, though other thicknesses are allowed.

[0093] Another aspect of the present disclosure is directed to a method of delivering a therapeutic agent to select cells or tissue of a subject. This method involves providing the composition of vitreous humor extracellular vesicles and/or aqueous humor extracellular vesicles modified to contain a therapeutic agent, and administering the composition to the subject under conditions effective to deliver the composition comprising the vitreous and/or aqueous humor extracellular vesicles modified to contain the therapeutic agent to the select cells or tissue of the subject.

[0094] In accordance with this aspect of the present invention, suitable subjects include any mammalian subject. Typically, the subject is human, however, non-human mammals amenable to receiving the composition of extracellular vesicles as described herein include non-human primates, dogs, cats, rodents (*e.g.*, mouse, rat, guinea pig), horses, cervids, cattle and cows, sheep, and pigs.

[0095] In one embodiment, the composition of extracellular vesicles is an autologous composition, *i.e.*, the extracellular vesicles of the composition were isolated from ocular fluids, *i.e.*, vitreous humor and/or aqueous humor of the same subject being administered the composition. In another embodiment, the composition is an allogenic composition where the

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donor subject that provided the ocular fluids containing the extracellular vesicles and the recipient subject to be treated are the same species but different individuals. In an alternative embodiment, the composition may be xenogenic. In this embodiment, the vitreous and/or aqueous humor vesicles are obtained from a donor subject that is a different species than the recipient species. For example, bovine extracellular vesicles may be isolated and modified to produce a composition suitable for treating a human subject.

[0096] In one embodiment, the subject being administered the composition of extracellular vesicles described herein is a subject having an ocular disease, and the administration of the composition delivers the therapeutic agent to the subject's ocular cells or tissue as a treatment for the ocular disease.

[0097] Ocular diseases that can be treated via administration of the compositions described herein include, without limitation, ocular degenerative diseases, such as dry macular degeneration, macular edema secondary to vascular disorders, retinitis pigmentosa, and wet macular degeneration; all forms of glaucoma, open-angle glaucoma (e.g., low tension and normal tension glaucoma), angle-closure glaucoma, congenital glaucoma, secondary glaucoma, neovascular glaucoma, pigmentary glaucoma, primary juvenile glaucoma, pseudoexfoliation glaucoma, irido corneal endothelial syndrome, and glaucoma of miscellaneous origin (e.g., glaucoma associated with intraocular tumors, retinal detachments, chemical burns, iris atrophy, and toxic glaucoma); inflammatory diseases, such as birdshot retinopathy, diabetic retinopathy, Harada's and Vogt-Koyanagi-Harada syndrome, iritis, multifocal choroiditis and panuveitis, pars planitis, posterior scleritis, sarcoidosis, retinitis due to systemic lupus, erythematosus, sympathetic ophthalmia, subretinal fibrosis, uveitis syndrome, white dot syndrome; ocular disorders associated with neovascularization, including age-related macular degeneration, angioid streaks, branch retinal vein occlusion, choroiditis, corneal trauma-related disorders, diabetes-related iris neovascularization, diabetic retinopathy, idiopathic choroidal neovascularization, pathologic myopia, retinal detachment, retinal tumors, retinopathy of prematurity, and sickle cell retinopathy; ocular infections associated with the choroids, retina, or cornea, such as, cytomegalovirus retinitis, histoplasma, retinochoroiditis, toxoplasma, retinochoroiditis, and tuberculous choroiditis; neoplastic diseases such as abnormal tissue growth in the retina, choroid, uvea, vitreous or cornea, choroidal melanoma, intraocular lymphoma of the choroids, vitreous, or retina, metastatic lesions, retinoblastoma, and vitreous seeding from retinoblastoma; and trauma, such as trauma resulting from injury or surgery or retinal damage resulting from exposure to laser or intense light.

[0098] Particular disorders of the corneal that are suitable for treatment using the methods and compositions described herein include corneal abrasion, corneal dystrophy, corneal ulcer,

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corneal neovascularization, fuchs' dystrophy, keratitis, keratoconus, allergic conjunctivitis, dry eye syndrome, dry eye, rheumatoid arthritis, sjögren's syndrome, problems following keratoplasty, corneal injury, allergies, bacterial keratitis, viral keratitis, herpes simplex virus (hsv) infections, and the varicella-zoster virus (vzv) causing herpes zoster, ophthalmicus, fungal

5 keratitis (keratomycosis), protozoal keratitis, acanthamoeba, megalocornea, microcornea, cornea plana, keratoglobus, corneal opacities, marginal keratitis, rosacea, keratitis, ulcerative keratitis, pterygium, mooren's ulcer, dellen, phlyctenulosis, terrien's marginal degeneration, arcus senilis, vogt's limbal girdle, cornea guttata, lipid keratopathy, band keratopathy, spheroidal degeneration, salzmann's nodular degeneration, crocodile shagreen, fuchs' endothelial dystrophy, lattice

10 dystrophy, map-dot-fingerprint dystrophy, pellucid marginal degeneration, keratoglobus, iridocorneal endothelial (ice) syndrome, exposure keratopathy, astigmatism, drug-induced keratopathies, thygeson's superficial punctate keratopathy, cystinosis, immunoprotein deposits, mucopolysaccharidoses, and wilson's disease.

[0099] Disorders of the conjunctiva that are suitable for treatment using the methods and

15 compositions described herein include, but are not limited, to acute conjunctivitis, acute atopic conjunctivitis, acute chemical conjunctivitis, chronic allergic conjunctivitis, other chronic allergic conjunctivitis, adenoviral conjunctivitis, viral conjunctivitis, conjunctivochalasis, conjunctival hemorrhage, pingueculum, pingueculitis, serous conjunctivitis.

[0100] Corneal dystrophies that are suitable for treatment using the methods and

20 compositions described herein include, but are not limited, endothelial (fuchs), granular, lattice, macular, other hereditary corneal dystrophies such as anterior basement membrane dystrophy and posterior polymorphous corneal dystrophy, avellino corneal dystrophy, macular corneal dystrophy, gelatinous drop-like dystrophy, schnyder corneal dystrophy, francois-neetans fleck dystrophy, congenital hereditary stromal dystrophy. Also included is corneal edema/ opacity/

25 degeneration, bullous keratopathy, corneal edema secondary to contact lens, diopathic corneal edema, secondary corneal edema, rupture in descemet's membrane, central corneal opacity, peripheral corneal opacity, other corneal scars and opacities, minor corneal opacity, arcus senilis, band keratopathy, keratomalacia, nodular corneal degeneration, peripheral corneal degeneration, keratoconus stable, keratoconus unstable, corneal ectasia, descemetocoele, corneal transplant,

30 corneal transplant rejection, corneal transplant failure, corneal transplant infection, other complications of corneal transplant. Also suitable for treatment using the methods and compositions described herein include, corneal foreign body/injury/ laceration, corneal foreign body, conjunctival foreign body, burn of cornea and conjunctival sac, injury of conjunctiva and corneal abrasion without foreign body, ocular laceration and rupture with prolapse or loss of

35 intraocular tissue, ocular laceration and rupture without prolapse or loss of intraocular tissue,

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contusion of eyeball and periocular tissues (*e.g.* traumatic hyphema), herpes simplex, herpes viral keratitis, herpes viral conjunctivitis, other herpes viral diseases, herpes zoster, zoster conjunctivitis, zoster keratitis, zoster scleritis, other herpes zoster, keratitis, central corneal ulcer, ring corneal ulcer, corneal ulcer with hypopyon, marginal corneal ulcer, mooren's corneal ulcer, mycotic corneal ulcer, perforated corneal ulcer, corneal abscess, filamentary, photokeratitis, punctate exposure keratoconjunctivitis keratoconjunctivitis, keratoconjunctivitis sicca, neurotrophic keratoconjunctivitis, sicca syndrome with keratoconjunctivitis, phlyctenular keratoconjunctivitis, interstitial keratitis (*e.g.* cogan syndrome), localized vascularization of cornea, dry eye, recurrent erosion of cornea, corneal disorder due to contact lens, sjogren's syndrome, sicca syndrome, pterygium, peripheral pterygium, stationary pterygium, progressive pterygium, recurrent pterygium.

[0101] Disorders of the lens including cataracts can also be treated with the methods and compositions described herein.

[0102] Neuro-ophthalmic conditions that can be treated with the methods and compositions described herein include, without limitation, blepharospasm, cranial nerve palsy, facial dystonias, giant cell / temporal arteritis, intracranial hypertension, ischemic optic neuropathy, multiple sclerosis, optic nerve tumors, optic neuritis. optic neuropathy, visual field defects and non-arteritic anterior ischemic optic neuropathy(naion).

[0103] Retinal diseases that can be treated with the methods and compositions described herein include, but are not limited to branch retinal vein occlusion, central retinal vein occlusion, central serous chorioretinopathy, choroidal detachment, complex retinal detachment, congenital x-linked retinoschisis, epiretinal membranes, familial exudative vitreoretinopathy, idiopathic juxtafoveal telangiectasis, infectious retinitis, intraocular lens dislocation, macular edema, macular hole, persistent fetal vasculature, polypoidal choroidal vasculopathy, posterior vitreous detachment, presumed ocular histoplasmosis syndrome, retained lens fragments, retinal artery occlusion, retinitis pigmentosa and retinal prosthesis, retinopathy of prematurity, river blindness/onchocerciasis, vitreomacular traction syndrome, retinoblastoma, macular pucker, macular hole, floaters, bietti's crystalline dystrophy, histoplasmosis, retinoblastoma, usher's syndrome.

[0104] Retinal disorders that can be treated with the methods and compositions described herein include, without limitation, diabetic retinopathy from diabetes mellitus type 1 with or without the following complications; without mention of complication, with mild non-proliferative retinopathy, with macular edema, with mild non-proliferative retinopathy, without macular edema, with moderate non-proliferative retinopathy, with macular edema, with moderate non-proliferative retinopathy, without macular edema, with severe non-proliferative

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retinopathy, with macular edema; with severe non-proliferative retinopathy, without macular edema, with proliferative retinopathy, with macular edema; with proliferative retinopathy, without macular edema. Diabetic retinopathy from diabetes mellitus type 2; without mention of complication, with mild non-proliferative retinopathy, with macular edema, with mild non-

5 proliferative retinopathy, without macular edema, with moderate non-proliferative retinopathy, with macular edema, with moderate non-proliferative retinopathy, without macular edema, with severe non-proliferative retinopathy, with macular edema, with severe non-proliferative retinopathy, without macular edema, with proliferative retinopathy, with macular edema, with proliferative retinopathy, without macular edema. Other disorders of the retina include

10 degeneration of macula and posterior pole type, nonexudative macular degeneration (dry), exudative macular degeneration (wet) macular cyst, hole, or pseudohole, central serous chorioretinopathy, cystoid macular degeneration (cme), puckering of macula (erm), drusen (degenerative) of macula, vitreomacular traction, cystoid macular edema following cataract surgery, degeneration of vitreous body type including vitreous hemorrhage, vitreous

15 degeneration (*e.g.*, pvd) vitreomacular adhesion (vmt), crystalline deposits in vitreous body, other vitreous opacities (*e.g.*, vitreous oaters), other disorders of vitreous body, disorders of optic nerve type including, coloboma of optic disc, drusen of optic disc, ischemic optic neuropathy, optic papillitis, other optic atrophy, papilledema associated with increased intracranial pressure, primary optic atrophy, retrobulbar neuritis, endophthalmitis, other endophthalmitis,

20 panophthalmitis (acute), panuveitis, purulent endophthalmitis, sympathetic uveitis. Hereditary retinal dystrophies, dystrophies primarily involving the retinal pigment epithelium, other dystrophies primarily involving the sensory retina (*e.g.*, staargardt's disease), pigmentary (*e.g.*, retinitis pigmentosa) dystrophies, vitreoretinal dystrophy, iridocyclitis, chronic iridocyclitis, lens induced iridocyclitis, primary iridocyclitis, recurrent acute iridocyclitis, secondary infectious

25 iridocyclitis, secondary noninfectious iridocyclitis, amaurosis fugax, atrophy of globe (*e.g.*, phthisis bulbi), cataract (lens) fragments in eye following cataract surgery degenerative myopia (*e.g.* malignant), diplopia (double vision), migraine with aura, not intractable; with status migrainosus migraine with aura, not intractable; without status migrainosus migraine with aura, intractable; with status migrainosus migraine with aura, intractable; without status migrainosus,

30 ocular pain, ophthalmoplegic migraine, not intractable, ophthalmoplegic migraine, intractable, other abnormal glucose (*e.g.*, prediabetes) other migraine, not intractable; with status migrainosus, other migraine, not intractable; without status migrainosus other migraine, intractable; with status migrainosus, other migraine, intractable; without status migrainosus, other long term (current) drug therapy, other visual disturbance (blurred vision), other subjective

35 visual disturbances (*e.g.*, visual halos) rheumatoid arthritis, sudden visual loss, transient visual

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loss, lupus erythematosus. macula scars of posterior pole (postinflammatory) (post-traumatic), solar retinopathy, chorioretinal inflammation, choroidal hemorrhage, choroidal rupture, benign neoplasm of choroid, chorioretinal scars after surgery for detachment, serous choroidal detachment, hemorrhagic choroidal detachment, hypertensive retinopathy, exudative retinopathy, 5 retinal micro-aneurysms, unspecified retinal neovascularization, unspecified other non-diabetic proliferative retinopathy retinal hemorrhage, retinal edema (*e.g.*, cotton wool spots) retinal ischemia, peripheral retinal degeneration type, lattice degeneration of retina, microcystoid degeneration of retina pavingstone, degeneration of retina, age-related reticular degeneration of retina, secondary vitreoretinal degeneration, retinal detachments, retinal detachment with single 10 break, retinal detachment with multiple breaks, retinal detachment with giant retinal tear, retinal detachment with retinal dialysis, total retinal detachment, other retinal detachments, traction detachment of retina (*e.g.*, PVR w/ retinal detachment) unspecified, retinoschisis, other retinoschisis and retinal cysts, serous retinal detachment, serous retinal detachment, retinal tear type, retinal break, horseshoe tear of retina without detachment, round hole of retina without 15 detachment, multiple defects of retina without detachment.

[0105] All types of retinal vascular occlusions can also be treated with the methods and compositions described herein, including central retinal artery occlusion (crao), retinal artery branch occlusion (brao), central retinal vein occlusion (crvo), tributary (branch) retinal vein occlusion (brvo), retinopathy of prematurity (ROP) type retinopathy of prematurity, ROP stage 20 0, retinopathy of prematurity, stage 1, retinopathy of prematurity stage 2, retinopathy of prematurity stage 3, retinopathy of prematurity stage 4, retinopathy of prematurity stage 5. Separation of retinal layers, including central serous chorioretinopathy (csr), serous detachment of retinal pigment epithelium, and hemorrhagic detachment of retinal pigment epithelium can also be treated in accordance with the methods and compositions described herein.

25 **[0106]** Disorders of choroid and retina that can be treated with the methods and EV compositions described herein include, without limitation, chorioretinal inflammation, focal chorioretinal inflammation, focal chorioretinitis, choroiditis, retinitis, retinochoroiditis, disseminated chorioretinal inflammation disseminated: chorioretinitis, choroiditis, retinitis, retinochoroiditis, exudative retinopathy, posterior cyclitis, pars planitis, other chorioretinal 30 inflammations, harada's disease, chorioretinal inflammation, unspecified; chorioretinitis, choroiditis, retinitis, retinochoroiditis, chorioretinal scars, macula scars of posterior pole (postinflammatory) (post-traumatic), solar retinopathy, choroidal degeneration, choroidal atrophy, choroidal sclerosis, angioid streaks, hereditary choroidal dystrophy, choroideremia, dystrophy, choroidal (central areolar) (generalized) (peripapillary), gyrate atrophy, choroid 35 ornithinaemia, choroidal haemorrhage and rupture, choroidal haemorrhage not otherwise

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specified, expulsive choroidal detachment, other specified disorders of choroid, chorioretinal disorders in diseases classified elsewhere, chorioretinal disorders in diseases classified elsewhere, chorioretinal inflammation in infectious and parasitic diseases classified elsewhere, chorioretinitis: syphilitic, late, toxoplasma, tuberculous, other chorioretinal disorders in diseases
 5 classified elsewhere, retinal detachments and breaks, retinal detachment, retinoschisis (including x-linked retinoschisis), retinal artery occlusion, retinal vein occlusion, hypertensive retinopathy, age-related macular degeneration, macular degeneration, epiretinal membrane, peripheral retinal degeneration, hereditary retinal dystrophy, retinitis pigmentosa, central serous retinopathy, retinal detachment, detachment of retinal pigment epithelium, other specified retinal disorders,
 10 macular edema, retinal disorder, unspecified, retinal disorders in diseases classified elsewhere.

[0107] Disorders of eyelid and lacrimal system and orbit that can also be treated with the methods and compositions as described herein include, without limitation, ectropion, lagophthalmos, blepharochalasis, ptosis, chalazion, hordeolum, xanthelasma of eyelid, parasitic infestation of eyelid in diseases classified elsewhere, dermatitis of eyelid due to demodex
 15 species, parasitic infestation of eyelid including, leishmaniasis, loiasis, onchocerciasis, phthiriasis, involvement of eyelid in other infectious diseases classified elsewhere. Involvement of eyelid in herpesviral (herpes simplex) infection, leprosy, molluscum contagiosum, tuberculosis, herpes zoster, involvement of eyelid in other diseases classified elsewhere, involvement of eyelid in impetigo, acryoadenitis, epiphora, dysthyroid exophthalmos, thyroid
 20 eye disease,

[0108] Glaucoma disorders that can be treated with the methods and compositions as described herein include, but are not limited to, preglaucoma open angle with borderline findings, open angle, low risk, anatomical narrow angle primary angle closure suspect, steroid responder, ocular hypertension, primary angle closure without glaucoma damage (pas or high iop
 25 with no optic nerve or visual field loss), unspecified open-angle glaucoma, primary open-angle glaucoma, chronic simple glaucoma, low-tension glaucoma, pigmentary glaucoma, capsular glaucoma with pseudo-exfoliation of lens, residual stage of open-angle glaucoma, unspecified primary angle-closure glaucoma, acute angle-closure glaucoma attack, chronic angle-closure glaucoma, intermittent angle-closure glaucoma, residual stage of angle-closure glaucoma,
 30 glaucoma secondary to eye trauma, glaucoma secondary to eye inflammation, glaucoma secondary to other eye disorders including, retinal vascular occlusions, diabetes type 1 complicated, diabetes type 2 complicated, disorders of lens, disorders of intraocular lens, disorders after other ocular symptoms, neoplasms, benign neoplasms, or malignant. Also included is glaucoma secondary to drugs, glaucoma with increased episcleral venous pressure,
 35 hypersecretion glaucoma, aqueous misdirection malignant glaucoma, glaucoma in diseases

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classified elsewhere, congenital glaucoma, axenfeld's anomaly, buphthalmos, glaucoma of childhood, glaucoma of newborn, hydrophthalmos, keratoglobus, congenital glaucoma macrocornea with glaucoma, macrophthalmos in congenital glaucoma, megalocornea with glaucoma, absolute glaucoma. Also included are adverse effect of ophthalmological drugs and preparations, acute follicular conjunctivitis, adverse effect of carbonic anhydrase inhibitors, and adverse effect of under dosing of ophthalmological drugs and preparations.

[0109] Disorders of optic nerve that can be treated with the methods and compositions as described herein include, but are not limited to, glaucomatous optic atrophy, optic papillitis, retrobulbar neuritis, unspecified optic atrophy, primary optic atrophy, unspecified optic neuritis, other optic neuritis, pseudopapilledema of optic disc, unspecified papilledema, papilledema, ischemic optic neuropathy, disorders of optic chiasm, disorders of optic chiasm associated with other neoplasms, disorders of optic chiasm associated with vascular disorders, disorders of optic chiasm associated with inflammatory disorders, other disorders of optic nerve, compression of optic nerve, toxic optic neuropathy, nutritional optic neuropathy, hereditary optic atrophy, cortical blindness, granuloma of orbit (*e.g.* pseudotumor (inflammatory) of orbit), tonic pupil, benign neoplasm of pituitary gland, benign neoplasm of unspecified site of orbit, anisocoria, conversion disorder with sensory symptom, benign neoplasm of cerebral meninges, ocular pain, thyrotoxicosis with diffuse goiter with thyrotoxic crisis or storm (*e.g.* graves' disease, exophthalmic or toxic goiter not otherwise specified), thyrotoxicosis with diffuse goiter without thyrotoxic crisis or storm (*e.g.* graves' disease, exophthalmic or toxic goiter not otherwise specified), mydriasis, anisocoria, other specified disorders of binocular movement (*e.g.* skew deviation), convergence insufficiency internuclear ophthalmoplegia, other giant cell arteritis, tonic pupil, other subjective visual disturbances (*e.g.* visual halos), elevated erythrocyte sedimentation rate, cerebral infarction, (*e.g.* stroke) transient cerebral ischemic attack, malignant neoplasm of orbit, progressive external ophthalmoplegia, focal chorioretinal inflammation, juxtapapillary, acquired color vision deficiency, scotoma of blind spot, partial retinal artery occlusion (*e.g.* hollenhorst's), palsy (spasm) of conjugate gaze, diplopia (double vision) other strabismus type, esophoria, exophoria, vertical strabismus (*e.g.* hypertropia), palsies type, third ocular motor nerve, fourth ocular motor nerve, sixth ocular motor nerve, ptosis, congenital ptosis, mechanical ptosis, myogenic ptosis, paralytic ptosis, visual field disturbances, transient visual loss (*e.g.* scintillating scotoma), homonymous bilateral visual field defects, heteronymous bilateral field defects.

[0110] Disorders of the nervous system that can be treated with the methods and compositions as described herein include, but are not limited to, amaurosis fugax, horner's syndrome, blepharospasm, multiple sclerosis, transient cerebral ischemic attack, benign

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intracranial hypertension, ophthalmoplegic migraine, not intractable, ophthalmoplegic migraine, intractable, myasthenia gravis without (acute) exacerbation, myasthenia gravis with (acute) exacerbation, clonic hemifacial spasm.

[0111] Other conditions amenable to treatment with the composition of vitreous and/or aqueous humor extracellular vesicles modified to contain a therapeutic agent include as described herein, without limitation, hematological malignancies, cutaneous T-cell lymphoma, adult T-cell lymphoma/leukemia, pathologic fibrosis, cutaneous fibrosis, idiopathic pulmonary fibrosis, other fibrotic indications, neurodegeneration, ischemia, acute intermittent porphyria, solid cancer, liver cancer, adrenocortical carcinoma, pancreatic cancer, hypercholesterolemia, diabetic macular edema, acute nonarteritic anterior ischemic optic neuropathy, prevention of acute kidney injury, delayed graft function in kidney transplant recipients, familial amyloid polyneuropathy, advanced cancer, elevated triglycerides, amyotrophic lateral sclerosis, prostate cancer, myelodysplastic syndrome, Huntington's disease, elevated triglycerides/familial hypercholesterolemia, solid cancer, cystic fibrosis, ulcerative colitis, solid cancer, duchenne muscular dystrophy, hyperlipoproteinemia(a), hepatitis B infection, type 2 diabetes, allergen-induced asthma, asthma, atopic dermatitis, liquid cancer, myeloid leukemia, clotting disorders, pouchitis, familial chylomicronemia syndrome, familial partial lipodystrophy, familial amyloid polyneuropathy, prostate cancer, non small cell lung cancer, melanoma, triple negative breast cancer, rabies, RSV, HIV, influenza A, cardiovascular disease, zika, prostate cancer, multiple myeloma, acute myeloid leukemia, non-small cell lung cancer, renal cell carcinoma, solid cancer, Pachyonychia congenita, liver fibrosis, Primary hyperoxaluria type 1, hypertrophic scarring, severe hemophilia A or B, paroxysmal nocturnal hemoglobinuria, liver and lung disease, hemophilia and rare bleeding disorders, hypercholesterolemia, acutes hepatic porphyrias, complement mediated diseases, primary hyperoxaluria type 1, hereditary ATTR amyloidosis, hepatitis B and C virus infection, HCV, AMD/DME, AMD, NAION, Pachyonychia Congenita, FAP/ colon cancer, PDAC, CML, AKI and DGF.

[0112] In accordance with this embodiment of the present disclosure, the extracellular vesicles of the composition are modified to contain one or more therapeutic agents that are suitable for treating the ocular disease. Suitable therapeutic agents, *i.e.*, nucleic acid molecules (therapeutic RNAs and DNAs), protein and peptide therapeutics, and small molecule therapeutics are described *supra*. The selection of a suitable therapeutic for a particular ocular disease is well within the level of skill of a person of skill in the field of ophthalmology.

[0113] In accordance with this aspect of the present disclosure, the composition containing the vitreous and/or aqueous humor extracellular vesicles can be administered to a subject in need thereof using topical administration, systemic administration, periocular

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administration, or intraocular administration. The particular route of administration selected is dependent on the condition being treated and formulation of the composition.

[0114] In one embodiment, the composition is administered systemically. Systemic administration can be achieved via intravenous administration, oral administration, intraarterial administration, inhalation, intranasal administration, intra-peritoneal administration, intra-abdominal administration, subcutaneous administration, intra-articular administration, intrathecal administration, transdural administration, transdermal administration, submucosal administration, sublingual administration, enteral administration, parenteral administration, percutaneous administration, periarticular administration, or intraventricular administration.

[0115] In another embodiment, the composition is administered locally. In one embodiment, the composition is administered locally to ocular tissue. As referred to herein, ocular tissue refers to the eye, including tissues within the sclera (*e.g.*, the retina) and outside the sclera (*e.g.*, ocular muscles within the orbit). Ocular tissue also includes tissues neurologically connected to (but distinct from) the eye, such as the optic nerve, the geniculate nucleus and the visual cortex. Local administration to ocular tissue can be achieved via intraocular administration. In accordance with this embodiment, intraocular administration can be carried out via intracameral administration, intravitreal administration, or subretinal administration.

[0116] In another embodiment, local administration to ocular tissue can be achieved via periocular administration. Periocular administration can be carried out via sub-conjunctival injection, sub-Tenon's injection, direct periocular injection, or depot periocular injection.

[0117] The target cells and/or tissue of the extracellular vesicles can include any desired cell and/or tissue type. In one embodiment, the target cells are ocular cells. Suitable ocular cells for delivery of the therapeutic agent via the extracellular vesicles as described herein include, without limitation, ciliary epithelium, pigmented ciliary epithelium, non-pigmented ciliary epithelium, ciliary processes, retinal cells including Müller cells, ganglion cells, amacrine cells, horizontal cells, photoreceptors (rods and cones) bipolar cells, retinal pigment epithelium or retinal endothelial cells, cells of the cornea including corneal epithelium, corneal stroma (keratocytes), corneal endothelium, or limbal stem cells, cells of iris including pigmented or non-pigmented cells, spindle shaped fibroblasts, macrophages (clump cells of Koganei), smooth muscle of the sphincter muscle, or posterior epithelium, trabecular meshwork cells including trabecular meshwork cells or endothelial cell lining of Schlemm's canal, cells of the lens including lens epithelium, anterior lens epithelial cell, crystallin-containing lens fiber cell, lens fibers, or lens capsule, cells of choroid including cuboidal epithelial cells, ependymal cell layer, choroid plexus epithelial cells, or choroidal endothelial cells, cells of the optic nerve including

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oligodendrocytes, retinal ganglion cell axons, or glial cells, stem and progenitor cells including mesenchymal stem cells, limbal stem cells, retina stem cells.

[0118] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include non-ocular cells. Non-ocular target cells and tissue for therapeutic delivery using the extracellular vesicles as described herein include, without limitation, exocrine secretory cells and tissue including but not limited to, epithelial cells, salivary gland mucous cell (polysaccharide-rich secretion), salivary gland number 1 (glycoprotein enzyme-rich secretion), von ebner's gland cell in tongue (washes taste buds), mammary gland cell (milk secretion), lacrimal gland cell (tear secretion), ceruminous gland cell in ear (earwax secretion), eccrine sweat glander dark cell (glycoprotein secretion), eccrine sweat gland clear cell (small molecule secretion), apocrine sweat gland cell (odoriferous secretion, sex-hormone sensitive), gland of moll cell in eyelid (specialized sweat gland), sebaceous gland cell (lipid-rich sebum secretion), bowman's gland cell in nose (washes olfactory epithelium), brunner's gland cell in duodenum (enzymes and alkaline mucus), seminal vesicle cell (secretes seminal fluid components, including fructose for swimming sperm), prostate gland cell (secretes seminal fluid components), bulbourethral gland cell (mucus secretion), bartholin's gland cell (vaginal lubricant secretion), gland of litre cell (mucus secretion), uterus endometrium cell (carbohydrate secretion), insolated goblet cell of respiratory and digestive tracts (mucus secretion), stomach lining mucous cell (mucus secretion), gastric gland zymogenic cell (pepsinogen secretion), gastric gland oxyntic cell (hydrochloric acid secretion), pancreatic acinar cell (bicarbonate and digestive enzyme secretion, paneth cell of small intestine (lysozyme secretion), type ii pneumocyte of lung (surfactant secretion), club cell of lung.

[0119] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include hormone-secreting cells including but not limited to, anterior pituitary cells, somatotropes, lactotropes, thyrotropes, gonadotropes, corticotropes, intermediate pituitary cell, secreting melanocyte-stimulating hormone, magnocellular neurosecretory cells, nonsecreting oxytocin, secreting vasopressin, gut and respiratory tract cells, secreting serotonin, secreting endorphin, secreting somatostatin, secreting gastrin, secreting secretin, nonsecreting cholecystokinin, secreting insulin, secreting glucagon, nonsecreting bombesin, thyroid gland cells, thyroid epithelial cell, parafollicular cell, parathyroid gland cells, parathyroid chief cell, oxyphil cell, adrenal gland cells, chromaffin cells, secreting steroid hormones (mineralocorticoids and gluco corticoids), leydig cell of testes secreting testosterone, theca interna cell of ovarian follicle secreting estrogen, corpus luteum cell of ruptured ovarian follicle secreting progesterone, granulosa lutein cells, theca lutein cells, juxtaglomerular cell (renin secretion), macula densa cell of kidney, peripolar cell of kidney, mesangial cell of kidney,

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pancreatic islets (islets of langerhans), alpha cells (secreting glucagon), beta cells (secreting insulin and amylin), delta cells (secreting somatostatin), pp cells (gamma cells) (secreting pancreatic polypeptide), epsilon cells (secreting ghrelin).

[0120] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include cells derived primarily from ectoderm including cells from the integumentary system, keratinizing epithelial cells, epidermal keratinocyte (differentiating epidermal cell), epidermal basal cell (stem cell), keratinocyte of fingernails and toenails, nail bed basal cell (stem cell), medullary hair shaft cell, cortical hair shaft cell, cuticular hair shaft cell, cuticular hair root sheath cell, hair root sheath cell of huxley's layer, hair root sheath cell of henle's layer, external hair root sheath cell, hair matrix cell (stem cell), wet stratified barrier epithelial cells, surface epithelial cell of stratified squamous epithelium of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, basal cell (stem cell) of epithelia of cornea, tongue, oral cavity, esophagus, anal canal, distal urethra and vagina, urinary epithelium cell (lining urinary bladder and urinary ducts).

[0121] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include nervous system cells including but not limited to sensory transducer cells, auditory inner hair cell of organ of corti, auditory inner hair cell of organ of corti, auditory outer hair cell of organ of corti, basal cell of olfactory epithelium (stem cell for olfactory neurons), cold-sensitive primary sensory neurons, heat-sensitive primary sensory neurons, merkel cell of epidermis (touch sensor), olfactory receptor neuron, pain-sensitive primary sensory neurons (various types), photoreceptor cells of retina in eye:, photoreceptor rod cells, photoreceptor blue-sensitive cone cell of eye, photoreceptor green-sensitive cone cell of eye, photoreceptor red-sensitive cone cell of eye, proprioceptive primary sensory neurons (various types), touch-sensitive primary sensory neurons (various types), type i carotid body cell (blood ph sensor), type ii carotid body cell (blood ph sensor), type i hair cell of vestibular system of ear (acceleration and gravity), type ii hair cell of vestibular system of ear (acceleration and gravity), type i taste bud cell, autonomic neuron cells, cholinergic neural cell (various types), adrenergic neural cell (various types), peptidergic neural cell (various types), sense organ and peripheral neuron supporting cells, inner pillar cell of organ of corti, outer pillar cell of organ of corti, inner phalangeal cell of organ of corti, outer phalangeal cell of organ of corti, border cell of organ of corti, hensen cell of organ of corti, vestibular apparatus supporting cell, taste bud supporting cell, olfactory epithelium supporting cell, schwann cell, satellite glial cell (encapsulating peripheral nerve cell bodies), enteric glial cell, central nervous system neurons and glial cells, neuron cells (large variety of types, still poorly classified), interneurons, basket cells, cartwheel cells, stellate cells, golgi cells, granule cells, lugaro cells, unipolar brush cells,

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martinotti cells, chandelier cells, medium spiny neurons, cajal–retzius cells, double-bouquet cells, neurogliaform cells, spinal interneuron, renshaw cells, principal cells, spindle neuron, pyramidal cells, place cells, grid cells, speed cells, head direction cells, betz cells, stellate cells, boundary cells, astrocyte (various types), oligodendrocyte, ependymal cells, and tanycytes.

5 **[0122]** In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include cells derived primarily from mesoderm including but not limited to metabolism and storage cells, adipocytes:, white fat cell, brown fat cell, liver lipocyte, barrier function cells (lung, gut, exocrine glands and urogenital tract), kidney, kidney parietal cell, kidney glomerulus podocyte, kidney proximal tubule brush border cell, loop of henle thin
10 segment cell, kidney distal tubule cell, kidney collecting duct cell, principal cells, intercalated cells, other, type i pneumocyte (lining air space of lung cell), pancreatic duct cell (centroacinar cell), nonstriated duct cell (of sweat gland, salivary gland, mammary gland, etc.), principal cell, intercalated cell, duct cell (of seminal vesicle, prostate gland, etc.), intestinal brush border cell (with microvilli), exocrine gland striated duct cell, gall bladder epithelial cell, ductulus efferens
15 nonciliated cell, epididymal principal cell, epididymal basal cell, endothelial cells.

[0123] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include extracellular matrix cells including but not limited to ameloblast epithelial cell (tooth enamel secretion), planum semilunatum epithelial cell of vestibular system of ear (proteoglycan secretion), organ of corti interdental epithelial cell
20 (secreting tectorial membrane covering hair cells), loose connective tissue fibroblasts, corneal fibroblasts (corneal keratocytes), tendon fibroblasts, bone marrow reticular tissue fibroblasts, other nonepithelial fibroblasts, pericyte, nucleus pulposus cell of intervertebral disc, cementoblast/cementocyte (tooth root bonelike ewan cell secretion), odontoblast/odontocyte (tooth dentin secretion), hyaline cartilage chondrocyte, fibrocartilage chondrocyte, elastic
25 cartilage chondrocyte, osteoblast/osteocyte, osteoprogenitor cell (stem cell of osteoblasts), hyalocyte of vitreous body of eye, stellate cell of perilymphatic space of ear, hepatic stellate cell (ito cell), pancreatic stelle cell.

[0124] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include contractile cells including but not limited to skeletal muscle
30 cell, red skeletal muscle cell (slow), white skeletal muscle cell (fast), intermediate skeletal muscle cell, nuclear bag cell of muscle spindle, nuclear chain cell of muscle spindle, satellite cell (stem cell), heart muscle cells, ordinary heart muscle cell, nodal heart muscle cell, purkinje fiber cell, smooth muscle cell (various types), myoepithelial cell of iris, myoepithelial cell of exocrine glands.

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[0125] In another embodiment, the target cells and/or tissue of the extracellular vesicles carrying a therapeutic agent include blood and immune system cells including but not limited to erythrocyte (red blood cell), megakaryocyte (platelet precursor), monocyte (white blood cell), connective tissue macrophage (various types), epidermal langerhans cell, osteoclast (in bone), dendritic cell (in lymphoid tissues), microglial cell (in central nervous system), neutrophil granulocyte, eosinophil granulocyte, basophil granulocyte, hybridoma cell, mast cell, helper T cell, suppressor T cell, cytotoxic T cell, natural killer T-cell, B-cell, natural killer cell, reticulocyte, stem cells and committed progenitors for the blood and immune system (various types), germ cells including but not limited to oogonium/oocyte, spermatid, spermatocyte, spermatogonium cell (stem cell for spermatocyte), spermatozoon, nurse cells including but not limited to ovarian follicle cell, sertoli cell (in testis), thymus epithelial cell, and interstitial cells including interstitial kidney cells.

[0126] In accordance with this aspect of the present disclosure, a subject is administered a therapeutically effective amount of the composition. A therapeutically effective amount is the amount effective to alleviate, inhibit, lessen, delay, and/or prevent at least one symptom or other aspect of the condition being treated. In another embodiment, a therapeutically effective amount is the amount effective to ameliorate the ocular condition being treated. The dose may be determined according to various parameters, especially according to the severity of the condition, age, and weight of the patient to be treated; the route of administration; and the required regimen. A physician will be able to determine the required route of administration and dosage for any particular patient. Optimum dosages may vary depending on the relative potency of the composition being administered, and can generally be estimated based on the half maximal effective concentration (EC50) found to be effective in in vitro and in vivo models. In general, dosage is from 0.01 mg/kg to 100 mg per kg of body weight. A typical daily dose is from about 0.1 to 50 mg per kg, preferably from about 0.1 mg/kg to 10 mg/kg of body weight, according to the potency of the specific construct, the age, weight and condition of the subject to be treated, the severity of the disease and the frequency and route of administration. Different dosages of the construct may be administered depending on whether administration is by systemic administration or local administration.

[0127] Due to therapeutic agent clearance (and breakdown of any targeted therapeutic molecule), the subject may have to be treated repeatedly, for example once or more daily, weekly, monthly or yearly. Persons of ordinary skill in the art can easily estimate repetition rates for dosing based on measured residence times and concentrations of the construct in bodily fluids or tissues. Following successful treatment, it may be desirable to have the patient undergo

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maintenance therapy, wherein the construct is administered in maintenance doses, ranging from 0.01 mg/kg to 100 mg per kg of body weight, once or more daily, monthly, yearly, etc.

[0128] Another aspect of the present disclosure is directed methods of making the composition comprising vitreous and/or aqueous humor extracellular vesicles as described

5 herein. An exemplary method involves providing a mammalian ocular fluid sample comprising vitreous and/or aqueous humor fluids, and isolating vesicular bodies from the ocular fluid sample. The method further involves inserting the one or more exogenous therapeutic agents into the isolated vesicular bodies.

[0129] In one embodiment, the ocular fluid sample is a human ocular fluid sample. In

10 another embodiment, the ocular fluid sample is a bovine ocular fluid sample. In another embodiment the ocular fluid sample is non-human mammalian ocular fluid sample, such as an ocular fluid sample obtained from a non-human primate, dog, cat, rodent, deer, sheep, pig, etc.

[0130] In one embodiment, the ocular fluid sample is a healthy, normal ocular fluid sample. In another embodiment, the ocular fluid sample is a diseased ocular fluid sample, or

15 obtained from a subject having an ocular disease or condition. Ocular fluid samples can be obtained using methods known in the art and described herein. In one embodiment, the ocular fluid sample is obtained via vitreous biopsy or an aqueous humor biopsy or aspiration.

[0131] Suitable methods of isolating extracellular vesicles from ocular fluids are described herein. In one embodiment, the method of isolating extracellular vesicles involves a series of centrifugation steps. As referred to herein, “ocular fluid” includes, without limitation, fluid from the vitreous humor, fluid from the aqueous humor, or any ocular fluid sample comprising the vitreous and/or aqueous humor fluid.

[0132] As described herein, the extracellular vesicles isolated from the aqueous humor and/or vitreous humor are modified to contain one or more exogenous agents. Methods of inserting the exogenous agent(s) into the extracellular vesicles can be achieved as described herein using methods and techniques readily known and practiced in the art, including, without limitation, electroporation, transfection, viral-vector delivery, or any combination thereof.

[0133] In one embodiment, the endogenous contents of the isolated extracellular vesicles are removed prior to inserting the one or more exogenous agents. Methods of removing the endogenous contents of the extracellular vesicles can be achieved using ultraviolet radiation. Other methods known in the art for emptying the contents of vesicular bodies are also suitable for use in accordance with this aspect of the present disclosure.

[0134] Another aspect of the present disclosure is directed to a method of identifying, detecting, diagnosing, monitoring, or prognosing an ocular disease in a subject. This method involves providing an ocular fluid sample that comprises vitreous and/or aqueous humor fluids

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from the subject, and isolating extracellular vesicles from the ocular fluid sample. This method further involves analyzing at least one molecular or physical property of the isolated extracellular vesicles, and comparing the at least one analyzed molecular or physical property of the isolated vesicular bodies to the molecular or physical property in isolated vesicular bodies obtained from a reference sample. The presence or absence of an ocular disease is identified, detected, or diagnosed based on that comparison. Alternatively, the comparison provides information regarding the progression or prognosis of the ocular disease or condition. A comprehensive list of ocular conditions that can be detected, diagnosed, and monitored based on the molecular and/or physical properties of the vitreous and/or aqueous humor extracellular vesicles is provided supra.

[0135] Described herein is the discovery of an extensive extracellular vesicle network in the normal, healthy vitreous humor and aqueous humor. A comprehensive proteomic analysis has been conducted to characterize the normal, healthy proteome of this extracellular vesicular network. Changes in this proteomic signature can be utilized as a means to identify, detect, diagnose, prognose, and/or monitor changes in ocular health in an individual. Similarly, other molecular properties of the isolated extracellular vesicles, such as, gene expression and lipid content of the extracellular vesicles in the sample obtained from normal, healthy ocular fluid can also be obtained, and utilized as reference values to track changes in ocular health of an individual overtime. Changes in gene expression and/or lipid content of the extracellular vesicles can be used to identify, detect, diagnose, prognose, and/or monitor changes in ocular health in an individual.

[0136] Accordingly, in one embodiment, an ocular fluid sample comprising aqueous and/or vitreous humor fluid is obtained from a healthy subject and the extracellular vesicles contained therein are isolated or purified. A proteomic, genomic, or lipid analysis is carried out to determine the subject's baseline or reference protein or gene expression signature or lipid content. Subsequently, a second ocular fluid sample comprising the aqueous and/or vitreous humor fluid is obtained, the extracellular vesicles of the aqueous humor and/or vitreous humor are isolated, and a protein expression, gene expression, and/or lipid content profile of the extracellular vesicles is determined. The second ocular fluid sample can be collected from the subject at any time after the first sample was collected. In one embodiment, the second sample is collected at or about the time the subject is experiencing one or more symptoms of an ocular condition. In other embodiment, the second sample is collected at a time that the subject has not yet experienced or exhibited any change in ocular health. The protein expression, gene expression, and/or lipid content of the first collected sample is compared to the protein expression, gene expression and/or lipid content of the second collected sample, respectively, to

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detect changes to one or more factors, i.e., protein expression, gene expression, and/or lipid content. Any changes in protein expression, gene expression, or lipid content are correlated to known changes in one or more ocular conditions to identify, detect, diagnose, and/or prognose the ocular health for the individual.

5 **[0137]** In another embodiment, changes in protein expression, gene expression, and/or lipid content are monitored in extracellular vesicle samples obtained from the aqueous and/or vitreous humor of a subject over time as a means of tracking progression (or lack of progression) of an ocular condition. In another embodiment, changes in protein expression, gene expression, and/or lipid content are monitored in extracellular vesicle samples obtained from the aqueous
10 and/or vitreous humor of a subject over time as a means of tracking or monitoring the effectiveness of a therapeutic intervention. Changes in protein or gene expression or lipid content overtime may indicate the effectiveness of the therapeutic intervention. Likewise, little or no change in protein or gene expression or lipid content over time may serve as an early indicator that the selected therapeutic intervention is ineffective in the monitored individual.
15 Such a finding may warrant a modification to the therapeutic intervention to improve effectiveness and treatment.

[0138] In addition to tracking and/or monitoring changes in one or more molecular properties, such as protein expression, gene expression, and/or lipid content for diagnostic, prognostic, or related purposes, one or more physical properties of the extracellular vesicles
20 derived from the vitreous and/or aqueous humor can be monitored in conjunction with or as an alternative to the one or more molecular properties. Suitable physical properties of the extracellular vesicles that can be measured and monitored include, without limitation, extracellular vesicle size, quantity, shape, and morphology. Methods of measuring such physical properties of extracellular vesicles derived from the vitreous and/or aqueous humor sample are
25 described herein.

[0139] The time between obtaining a first ocular extracellular vesicle sample and a second, or any additional subsequent ocular extracellular vesicle samples can be any desired period of time, for example, weeks, months, years, as determined is suitable by a physician and based on the characteristics of the ocular condition. In one embodiment, the first sample is
30 obtained before treatment and the second sample is obtained after treatment. Alternatively, both samples can be obtained after one or more therapeutic treatments; the second sample being obtained at some point in time later than the first sample.

EXAMPLES

[0140] The examples below are intended to exemplify the practice of the present invention but are by no means intended to limit the scope thereof.

Materials and Methods for Examples

5 [0141] **Tissue preparation and processing from post mortem samples.** Post-mortem human eyes without disease were obtained (The Eye-Bank for Sight Restoration, New York, NY). Bovine eyes were acquired from a local butcher shop (Green Village Packing, Green Village, New Jersey). For dissection procedures, eyes were placed in a 100 mm plastic petri dish on ice to prevent RNA and protein degradation. Using a SZX-16 stereo dissecting microscope
10 (Olympus) orbital fat and extraocular muscles attached to the globe were removed. The globe was rinsed with 5 ml of ice-cold Tris Buffered Saline (TBS) containing 50 mM Tris-HCl, 150 mM NaCl and the pH adjusted to 8.0 for 1 minute at 4°C. Vitreous was dissected by making an sclerotomy incision 4 mm or 8 mm posterior to the limbus (human and bovine eye, respectively) using a 16g needle and then making a circumferential sagittal incision with scissors to separate
15 the globe into an anterior and posterior cup. Scissors were used to cut and remove the formed vitreous and to sever adhesions between vitreous and ocular structures. Care was taken to avoid vitreous contamination of choroid melanocytes and the neural retina. Other ocular tissues including choroid, retina, ciliary body, lens and cornea were identified and dissected. Tissue samples were rinsed with TBS (pH 8.0) for 1 min at 4°C. Specimens collected for electron
20 microscopy and EV isolation were processed immediately without fixation as described below. Samples used for immunohistochemistry, western blot, or EDC-formalin fixation were placed in 15 ml centrifuge tubes and immersed in 10 ml of 4% formalin (also known as formaldehyde, paraformaldehyde, or PFA) diluted in TBS (pH 8.0) for at least 24 h at 4°C. Tissues that were “formalin only,” were washed three times in TBS (pH 8.0) for 5 min at 4°C and not further
25 processed or fixed with EDC. Formalin only tissues were used for immunohistochemistry, western blot or nucleic acid, and protein imaging. EDC-formalin fixed specimens were processed further as described below.

[0142] **Human subject surgical vitreous specimen collection.** Institutional Review Board (IRB) approval was obtained from Weill Cornell Medicine, and protocols were in
30 accordance with NIH guidelines, the Healthcare Insurance Portability and Accountability Act, and the tenets put forth by the Declaration of Helsinki. Informed consent was obtained from all subjects. Subjects were patients who were undergoing vitrectomy for an existing medical condition. Methods for vitreous biopsy were previously described (Malecaze et al., “Detection of Vascular Endothelial Growth Factor Messenger RNA and Vascular Endothelial Growth

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Factor-like Activity in Proliferative Diabetic Retinopathy,” *Arch Ophthalmol* 112:1476-1482 (1994), which is hereby incorporated by reference in its entirety). Briefly, at the beginning of pars plana vitrectomy, 0.5-1 ml of un-dilute vitreous (which is removed during vitrectomy surgery for medical purposes) was collected using the vitrectomy probe connected to a sterile 3-
 5 mL syringe for aspiration. All samples were de-identified and coded. The vitreous specimen was immediately placed on ice and transferred to the laboratory for TEM or vitreous vesicle isolation as described below.

[0143] EDC-formalin tissue fixation. Methods for EDC-formalin fixation were adapted from previous reports (Valadi et al., “Exosome-mediated Transfer of mRNAs and MicroRNAs is a Novel Mechanism of Genetic Exchange Between Cells,” *Nat Cell Biol* 9:654-659 (2007); Suzuki et al., “DNA Staining for Fluorescence and Laser Confocal Microscopy,” *J Histochem Cytochem* 45:49-53 (1997), which are hereby incorporated by reference in their entirety). A piece of vitreous (1 cm x 1 cm) was isolated as described and examined under the microscope to ensure the sample was free of contaminating tissues like retina or choroid. The tissue was placed
 15 into a 100 mm plastic petri dish and washed two times in 5 ml of TBS (pH 8.0) for 5 min at 4°C. The sample was immersed in 5 ml of 4% formalin diluted in TBS (pH 8.0) for 24 h and stored in a humidified chamber at 4°C. The samples were washed three times in ice-cold TBS (pH 8.0) for 5 min at 4°C. To remove residual phosphate from the tissue, the sample was incubated in 10 ml of a freshly prepared 0.1 M 1-Methylimidazole buffer solution (0.1 M 1-methylimidazole, 300 mM NaCl, with an adjusted pH to 8.0 with 12 N NaOH) for 30 min at 4°C. Next, the EDC
 20 fixation solution was prepared. First, 9.6 ml of 0.1 M 1-Methylimidazole buffer solution was made and 130 mg of 5-(Ethylthio)-1H-tetrazole (ETT, Sigma Aldrich, final concentration was 0.1 M) was added. The pH was adjusted to 8.0 with 12 N NaOH. Next, 192 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (Sigma Aldrich, final concentration 0.10 M) was added to the 1-Methylimidazole-ETT solution. The pH of the solution was readjusted as needed by addition of 12 M HCl to pH 8.0. The piece of vitreous tissue (1 cm x 1 cm) was then transferred to a 35 mm plastic petri dish and 2 ml of EDC fixation solution was added. The samples were then placed in a humidified chamber and specimens were incubated for 3 h at 37°C. After incubation, the EDC-ETT solution was removed and specimens were washed in 5
 30 ml of 0.2% (w/v) glycine diluted in TBS (pH 7.4). The samples were washed twice in TBS (pH 7.4). Finally, the samples were stained for DNA, RNA, and protein as described below.

[0144] Staining for DNA, RNA and protein. Vitreous tissues fixed with 4% formalin only or EDC-formalin as described above were stained. Tissues were then immersed with various dyes to label DNA, RNA or proteins. To mark DNA, a dissected piece of vitreous (1 cm
 35 x 1 cm) was placed in a 35 mm petri dish and immersed with 1 ml of 0.5 µg/ml of Hoechst

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33342 Stain Solution (Sigma Aldrich). Samples were incubated at 37°C for 15 min at room temperature and then tissues were washed with 5 ml of 1 x TBS (pH 7.4) for 3 min at room temperature. Wash steps were repeated twice. Samples were stained with secondary marker or mounted for imaging. To label both DNA and RNA with a single dye, propidium iodide (PI, Sigma Aldrich) was used, which intercalates between DNA bases and also binds to RNA, with less affinity (Le Goff and Bishop, "Adult Vitreous Structure and Postnatal Changes," *Eye (Lond)* 22:1214-1222 (2008), which is hereby incorporated by reference in its entirety). It was found that a solution of 50 µg/ml of PI diluted in TBS (pH 7.4) was the optimal concentration of PI for co-staining DNA and RNA in whole mounted vitreous samples. Therefore, tissues were placed in a 35 mm petri dish and then immersed in 1 ml solution 50 µg/ml of PI (diluted in TBS) for 24 h at 37°C in a humidified chamber. Samples were washed with TBS (pH 7.4) three times. Samples were stained with another marker or mounted for imaging. To differentiate between DNA and RNA, all tissues were co-stained with Hoechst 33342 Stain Solution. Hoechst has a strong affinity for DNA and does not label RNA. For Hoechst and PI stained samples, the RNA signal was determined by excluding the Hoechst signal. To label cellular and extracellular proteins in whole mount vitreous, the cell permeable and electron dense stain carboxyfluorescein succinimidyl ester (CFSE, Sigma Aldrich) was used, which covalently links to intracellular amines (Ikeda et al., "Extraction and Analysis of Diagnostically Useful Proteins From Formalin-fixed, Paraffin-embedded Tissue Sections," *J Histochem Cytochem* 46:397-403 (1998), and Tkach and Thery, "Communication by Extracellular Vesicles: Where We Are and Where We Need to Go." *Cell* 164:1226-1232 (2016), which are hereby incorporated by reference in their entirety). Vitreous tissues were placed in a 35 mm plastic petri dish and then tissues were immersed in 1 ml of 500 µM CFSE diluted in TBS (pH 7.4) and samples were incubated at 37°C for 24 h in a humidified chamber. After incubation, the CFSE solution was removed and the tissues were placed in a 100 mm plastic petri dish. The tissues were washed in 5 ml of 0.2% (w/v) glycine diluted in TBS (pH 7.4) for 30 min at room temperature. Next, tissues were washed in 10 ml of TBS (pH 7.4) for 5 min at room temperature and wash steps were repeated twice. Finally, samples were counterstained with Hoescht and or PI as described. After staining with the respective dye(s), the samples were then mounted in custom chambers for imaging on the multiphoton, confocal or wide-field fluorescent microscope as described below.

[0145] RNase digestion of extracellular RNA *in situ*. Vitreous tissues were fixed with EDC-formalin and immersed with 2 ml of RNase buffer (consisting of 50 mM Tris-Cl, pH 8.0, 10 mM EDTA) containing 100 µg / mL RNase A (Sigma Aldrich), and then incubated at 42°C for 16 hr. Next, the RNase solution was removed, samples washed, and stained with PI (described above), and imaged with wide-field fluorescent microscopy.

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[0146] Light microscopy, confocal microscopy, and image processing. Color bright field images were captured on a Nikon eclipse an upright e600 microscope (Nikon) equipped with an Axiocam 105 color camera (Zeiss), and images were processed with Zen software (Zeiss, version 4.3). Tissues were mounted on a 60 mm glass bottom dish (20 mm viewing area, MatTek) for fluorescent imaging studies. An Axio Observer Z1 inverted microscope (Zeiss) was used with the following filter sets: Ziess filter set 49 (Ziess) for Hoechst; Ziess filter set 38 (Ziess) for Alexa 488, green fluorescent protein (GFP), and fluorescein; and Ziess filter set 45 (Ziess) for PI. Confocal imaging was conducted with Zeiss LSM880 microscope using the 25x/0.8 NA lens. Images were captured and processed using imageJ software.

[0147] Multiphoton imaging. Bovine eyes were dissected as described above and vitreous was cut into sections that were approximately 1 cm x 1 cm. Tissues were fixed with EDC-formalin or formalin only as described. DNA, RNA, and/or protein were labeled with Hoechst, PI and/or CFSE as described above. Whole mount vitreous tissue was mounted on a specialized chamber made of silicone and a glass coverslip, and was placed on top of the chamber. The coverslip was immersed in 1 ml of 1 x TBS and then imaged using MPM (Olympus FV1000MPE, using a specialized 25x/1.05 NA water immersion objective, Weill-Cornell Medicine Imaging Core Facility). The vitreous was then imaged in sectors. The images were captured, z-stacks were assembled, and a 2-dimensional reconstruction was constructed (Fiji software (Schneider et al., "NIH Image to ImageJ: 25 years of image analysis," *Nat Methods* 9:671-675 (2012), which is hereby incorporated by reference in its entirety) and Imaris software (Bitplane), 6-regions imaged per vitreous, n = 3). The data was analyzed for staining of extracellular protein. EVs and vitreous cells were measured and counted.

[0148] Defining vitreous cells and extracellular vessels. The goal was to identify extracellular vesicles (EV) and extracellular RNA in the vitreous tissue. To do this, vitreous cells (presumed hyalocytes) and EVs were differentiated by the following method. Multiphoton or confocal images of EDC-formalin fixed bovine vitreous co-stained with Hoechst and CFSE were obtained as described above. Using these images, vitreous cells were identified by identifying the nuclei using the Hoechst signal and then the cell bodies were identified by using the CFSE signal. The diameter of the cell bodies was then measured from over 100 cell diameters (n = 3 biological samples, 6 image frames per sample) using ImageJ software (Schneider et al., "NIH Image to ImageJ: 25 years of image analysis," *Nat Methods* 9:671-675 (2012), which is hereby incorporated by reference in its entirety). The average vitreous cell body diameter and standard deviation (SD) was calculated and the data was presented graphically. It was found that the average vitreous cell size was $10.5 \mu\text{m} \pm 1.77 \mu\text{m}$ and normally distributed. Thus, an upper limit diameter of 2 SD above the mean ($14 \mu\text{m}$) would encompass approximately

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97.5% of cells. Therefore, in ImageJ software, a 14 μm circle centered on the nuclei was drawn, and considered positive signal within this circle as intracellular protein. Signal outside this 14 μm circle was considered to be extracellular. Two independent and blinded research assistants were used to count EVs. The criteria for counting EVs included round shape, location outside of the cell radius, and size larger than 100 nm and smaller than cells. The data was normalized by dividing the number of EVs counted per frame, by the number of cells in the frame. The data is represented graphically. The size of bovine vitreous EVs was also measured using similar techniques (n = 4, and 3 biological replicates).

[0149] Electron microscopy of vitreous humor, aqueous human, and ocular tissues.

Human or bovine vitreous tissue was obtained as above. Samples were cleared of cells with low speed centrifugation and whole mount specimens tested with H and E staining and imaging as described below. For vitreous, 2 μL was pipetted onto a block and fixed in a solution of 2.5% glutaraldehyde, 4% paraformaldehyde, 0.02% picric acid in 0.1M sodium cacodylate buffer and incubated at room temperature for 60 min (Raposo et al., "B Lymphocytes Secrete Antigen-presenting Vesicles," *J Exp Med* 183:1161-1172 (1996), which is hereby incorporated by reference in its entirety). Specimens were washed with excess volume of buffer (pH 7.3) for 5 minutes each at room temperature. Samples were post-fixed with 1% OsO_4 -1.5% K-ferricyanide (aqueous) for 60 min at room temperature (Griffith and Hay, "Epithelial-mesenchymal Transformation During Palatal Fusion: Carboxyfluorescein Traces Cells at Light and Electron Microscopic Levels," *Development* 116:1087-1099 (1992), which is hereby incorporated by reference in its entirety). Samples were washed with buffer 3 times for 5 min each at room temperature. Samples were set *en bloc* and stained with 1.5% uranyl acetate for 60 min at room temperature. Samples were dehydrated through graded ethanol series and transitioned through acetonitrile. Specimens were infiltrated and embedded in Embed 812 resin (Electron Microscopy Sciences). Tissue sections cut at 60-65 nm using a Diatome diamond knife (Diatome) on Leica Ultracut T ultramicrotome (Leica Microsystems). Sections were contrasted with lead citrate (Dragovic et al., "Sizing and Phenotyping of Cellular Vesicles Using Nanoparticle Tracking Analysis," *Nanomedicine* 7:780-788 (2011), which is hereby incorporated by reference in its entirety) and viewed on a JEM 1400 electron microscope (JEOL, USA, Inc) operated at 100kV. Digital images were captured on a Veleta 2K x 2K CCD camera (Olympus-SIS). Electron microscopy images were recorded and analyzed for size and frequency using ImageJ software.

[0150] For TEM visualization of whole mounts of extracellular vesicles from human or bovine vitreous were obtained after ultracentrifugation, re-suspended in formaldehyde, loaded on Formwar/carbon-coated EM grids, postfixed in 1% glutaraldehyde, and contrasted successively

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in 2% uranyl acetate, pH 7, and 2% methylcellulose/0.4% uranyl acetate, pH 4, or acridine orange or CFSE.

[0151] Extracellular vesicle isolation and purification. Methods for isolating extracellular vesicles from fluids (van der Pol et al., “Recent Developments in the Nomenclature, Presence, Isolation, Detection and Clinical Impact of Extracellular Vesicles,” *J Thromb Haemost* 14:48-56 (2016), which is hereby incorporated by reference in its entirety) were adapted. For this study, the goal was to have vitreous specimens free of cells. The vitreous was therefore cleared with a series of low-speed centrifugations. Approximately 8 ml of vitreous was placed in 15 ml tubes and centrifuged in Sorvall legend RT Swinging bucket (Sorvall) at 2,000 g (2500 rpm) at 4°C for 30 minutes. The supernatant was then transferred to a new 15 ml tube. Then the centrifugation step was repeated. The supernatant was then transferred to new tube and centrifuged at 10,000 g in a Sorvall RC-58 centrifuge (Sorvall) using an SS-34 rotor (DuPont) for 30 min at 4°C. For each aliquot of vitreous or aqueous humor, whole mount hematoxylin and eosin staining (H and E) was conducted to survey for cells as described below (FIG. 11). Whole mount slides were then imaged and all cell free samples were further processed. The supernatant was then transferred and the step was repeated. The sample was transferred to an ultracentrifuge tube (Beckman) and in a swinging bucket rotor (SW-41, Beckman) and centrifuged at 100,000 g in an L7-55 ultracentrifuge (Beckman) at 4°C for 1 hour. The supernatant was transferred to a new tube. The step was repeated. Samples were resuspended in 50 µl of sterile phosphate buffered saline (PBS, pH 7.5) and placed in a siliconized tube. Samples for imaging were immediately processed, and remaining sample was frozen at -80 °C.

[0152] Vitreous histochemical staining to confirm acellularity of samples. To optimize vitreous EV isolation techniques, histochemical stains were applied after low-speed centrifugation to exclude vitreous samples contaminated by cells. Vitreous samples were dissected and collected as above. Acellularity was confirmed by whole mounting centrifuged vitreous onto glass slides and then subjecting the specimen to histochemical staining with hematoxylin and eosin (H and E). Approximately 1 ml of vitreous supernatant was placed on SuperFrost Plus glass slides (Thermo Fisher Scientific) and then dried in a chamber for 16 hours at 4 °C. The dried slides were rinsed with 5 mls of 1 x TBS for 3 min at room temperature, and then washed again. The slides were then stained with H and E using standard procedures. Slides were preserved by mounting glass coverslips and then sealed. Samples were analyzed with light microscopy as described below. Specimens with hematoxylin-stained cells were subjected to repeat centrifugation or discarded. Therefore, all extracellular fractions used for further experiments were free from contaminating vitreous cells.

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[0153] Nanoparticle tracking analysis. The NanoSight NS300 system (Malvern) was used to perform nanoparticle tracking analysis to characterize particles from 30 – 800 nm in solution. Extracellular vesicles isolated from bovine vitreous were resuspended in 100 µl of phosphate buffered saline (PBS, pH 7.0) at a concentration of approximately 2.5 µg of protein per ml, and then the sample was diluted to a final volume of 2 ml in PBS for analysis. Particles were loaded, the camera was focused, and 5 videos were captured for 60 sec each. Videos were recorded and then analyzed using NanoSight software (Version 3.0) to determine the size distribution and particle concentration of EVs. Graphs were created. The Brownian motion of each particle is tracked between frames, ultimately allowing calculation of the size through application of the Stokes- Einstein equation.

[0154] Extracellular vesicle isolation from formalin-fixed tissue. Whole bovine vitreous microdissected as described above was placed in a 50 ml conical tube and then submerged in 10 ml of 4 % formalin diluted in TBS (pH 7.4) and incubated for 24 h at 4°C. After fixation, tissues were dissected on ice into approximately 1 cm x 1 cm sections and the weight of vitreous section was recorded. The tissues were then placed in 15 ml centrifuge tubes. The tissues were immersed in 250 µl of TBS and the sample and overlying wash buffer (or supernatant) was incubated at 37°C for 30 min, 1 hr, 3 hr, 6 hr and 24 hr (n = 3). The vitreous tissue and supernatant were collected and placed in separate 1.5 ml tubes for further protein studies. For the formalin fixed vitreous tissue, the specimen was homogenized at 4°C and then lysed in equal volume of NP-40 lysis buffer. The lysate was transferred to a 1.5 ml tube and centrifuged for 15 min at 12,000g at 4°C. The aqueous phase was transferred to a new tube without the white pellet. The protein pellet was collected by centrifugation for 15 min at 4°C at 12,000g and the supernatant removed. The pellet was then dissolved in 30 µl water and used for Western blotting. For the supernatant, the samples were cleared of cellular debris by centrifugation at 12,000g for 15 min at 4°C. The protein supernatant was collected lysed with equal volume of NP-40 lysis buffer. The lysate was used for Western blotting.

[0155] Western blotting. Vitreous tissue or vitreous supernatant (250 µl) was collected after incubation at designated times and temperatures. Vitreous supernatant was pre-cleared with centrifugation at 12,000g for 30 min at 4°C and then lysed in buffer (50mM Tris pH 8.0, 250 mM NaCl, 0.5% NP-40, protease inhibitors, Sigma Aldrich). An equal amount of protein (determined by BIO-RAD protein assay) from each sample was separated on SDS-PAGE gels, transferred to protein blotting membrane (Hybond, Amersham GE Healthcare), and blotted following standard procedures. The primary antibodies used included: rabbit monoclonal anti-TSG101 (Systems Bioscience). The monoclonal antibodies were blotted with a secondary

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antibody, IRDye 680LT Goat anti-Rabbit (LI-COR Inc.), followed by detection with fluorescent imaging system (Odyssey CLx, Li-Cor) according to manufacturers' recommendations.

[0156] Immunohistochemistry of exosome marker proteins in vitreous.

Immunohistochemistry was performed on whole mounted 4% formalin-fixed bovine vitreous.

- 5 To prevent formalin crosslinks from reverting and thus reduce the rate of EV loss, all were conducted at 4°C for the duration of the experiment, except for microscopic imaging. The bovine vitreous humor was cut into approximately 1 cm x 1 cm pieces and then the specimen was rinsed in 5 ml of ice-cold TBS (pH 7.4) for 3 minutes at 4°C. Wash steps were repeated twice. Specimens were then examined with a dissecting microscope (SZX-16 Olympus) to
- 10 remove potentially contaminating tissues. Samples were then immersed in 500 µl of blocking buffer (10% goat serum diluted in TBS) for 1 h at 4°C. The samples were briefly washed in 5 ml of TBS for 3 min at 4°C. The antibody to TSG-101 (System Biosciences, diluted 1:500) was used to immunostain the bovine vitreous overnight at 4°C. The samples were washed in 5 ml of TBS for 3 min at 4°C. Wash steps were repeated twice. IHC staining was visualized using a
- 15 secondary antibody, goat anti-rabbit IgG conjugated to Alexa Fluor 488 (Abcam). Samples were washed three times. Bovine vitreous was counterstained with Hoechst stain (as described above) to mark nuclei and then washed twice in 5 ml of TBS for 5 min at 4°C. The vitreous was then immediately imaged and photomicrographs were recorded. For negative controls, normal goat serum (1:1000 dilution) was substituted for the primary antibody (secondary antibody only).
- 20 Antibodies were verified to be specific for TSG101 using western blotting and the expected 45 kD protein band was observed.

[0157] Vitreous proteome analysis. Bovine vitreous samples were cleared of cells using the above protocol and whole mount samples were determined to be cell free by whole mount H and E staining and subsequent imaging as described above. Samples free of cells were

25 then selected for proteomic analysis. Protein from extracellular vesicle fraction or cell free vitreous fraction was denatured in 8M urea, and cysteines were reduced with dithiothreitol (Sigma Aldrich) prior to alkylation with iodoacetamide (Sigma Aldrich). Proteins were digested with LysC (Wako Chemicals) followed by trypsin (Promega) and desalted with Empore C18 STaGETips (3M) (Skog et al., "Glioblastoma Microvesicles Transport RNA and Proteins That

30 Promote Tumour Growth and Provide Diagnostic Biomarkers" *Nat Cell Biol* 10:1470-1476 (2008), which is hereby incorporated by reference in its entirety). One µg of total protein was injected for nano-LC-MS/MS analysis (Q-Exactive Plus, Thermo Scientific). Peptides were separated using a 12 cm x 75 µm C18 column (Nikkyo Technos Co., Ltd. Japan) at a flow rate of 200 nL / min, with a 5-40% gradient over 160 minutes (buffer A 0.1% formic acid, buffer B

35 0.1% formic acid in acetonitrile). The Q-Exactive Plus was operated in data-dependent mode,

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with a top 20 method. Nano-LC-MS/MS data were analyzed using MaxQuant (version 1.5) and Perseus software (version 1.4) (Tyanova et al., "The Perseus Computational Platform for Comprehensive Analysis of (Prote)omics Data," *Nat Methods* 13(9):731-740 (2016), which is hereby incorporated by reference in its entirety), searching against a Uniprot *Bos taurus* database (downloaded July 14), allowing oxidation of methionine and protein N-terminal acetylation, and filtering at a 1% false discovery rate at the peptide and protein level. Proteins were quantified using iBAQ values. Protein enrichment was compared between vitreous extracellular vesicle fraction and cell free vitreous fraction.

[0158] Cell culture. Human retinal pigmented epithelial cells, ARPE-19 (ATCC) were cultured in DMEM:F12 medium (ThermoFisher Scientific) supplemented with 10% fetal bovine serum, penicillin, and streptomycin. All cells were incubated at 37 °C in 95% air and 5% CO₂ and maintained using standard sterile techniques.

[0159] Loading recombinant proteins into EVs. Bovine vitreous EVs were obtained as described above and the total protein concentration was measured (Pierce™ BCA Protein Assay Kit, Thermo Fisher Scientific). 4 µg of vitreous EVs was used for *in vitro* treatments and 0.025 µg of bovine vitreous EVs was used for *in vivo* injections along with the following concentrations of BSA-fluorescein (3 µg, 1 µg, and 0.5 µg) or GFP (0.25 µg, 0.5 µg, and 1 µg). Recombinant protein and EVs were mixed in 300 µl of electroporation buffer (BioRad) and electroporated in a 4 mm cuvette. Electroporation was performed on the EVs using a square wave program under the following conditions; voltage at 300 V, pulse length time of 35 ms, with the number of pulses at 2, and pulse interval of 0.1 sec. For *in vitro* experiments, 100 µl of the electroporated solution was added to 300 µl of warm media, and the solution was transferred into each well of a 12-well plate (n = 3) with APRE-19 cells plated at 70% confluence. Cultures were incubated for 24 h and the media was then replaced with complete media. Cells were fixed with 4% paraformaldehyde and imaged at 48 h after treatment. For *in vivo* studies, electroporation was performed in 300 µl of electroporation buffer (BioRad) and electroporated in a 4 mm cuvette at 300 V. Samples were desalted after resuspension in balanced salt solution 5 volumes and then concentrated with centrifugal size exclusion filters (Amicon, Millipore Sigma). The re-suspension volume in balanced salt solution (BSS) was 75 µl and 0.5 µl was used per injection.

[0160] In vitro application of EVs to cultured cells. Bovine or post-mortem human vitreous EVs were isolated and loaded with recombinant protein via electroporation as described above. ARPE-19 cells were cultured on a 12-well plate and approximately 70% confluent at the time of EV treatment. Then, 100 µl of the electroporated EV solution was added to 1 ml of complete media. The cells were incubated for 16 h under standard culture conditions and then

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the media was removed and replaced with complete media. At 48 h post-treatment, cell media was removed and cultures immersed with 1 ml of Hoechst stain and incubated for 15 min at 37°C. The stain was removed and cells were washed with 2 ml of phosphate buffered saline and fixed with 2 mls of 4% formalin diluted in PBS for 10 min at room temperature. Cells were washed with 2 ml of PBS for 5 min. The wash was repeated twice. Cells were evaluated for transfection efficiency with using wide-field fluorescent microscopy.

[0161] In vivo injection of vitreous EVs. All procedures were performed in accordance with NIH guidelines and approved by Weill Cornell Medicine's Institutional Animal Care and Use Committee (IACUC). Male, 6-week-old C57BL/6J mice (Jackson Labs) were maintained on a 12-h light/dark cycle at Weill Cornell Medical College's Research Animal Resource Center (RARC). Intravitreal injections of mouse eyes occurred at 8 weeks of age in all experimental variables ($n \geq 3$). Animals were sedated with a ketamine and xylazine cocktail in accordance with NIH Animal Welfare guidelines. Pupils were dilated with 1 drop of 2.5% phenylephrine, 1 drop of 1% tropicamide, and then a lubricating ophthalmic ointment was applied. After 15 min, animals were prepared for injection. Ophthalmic ointment was removed using a cotton swab and eyes were rinsed with 10 drops of 1X TBS. Under a dissecting stereo microscope (Olympus SZX50), a guide track was made in the eye by positioning a 32-gauge needle at the limbus and then traversing from the sclera and into the posterior chamber. Care was taken to avoid disrupting the crystalline lens. Next, the guide needle was withdrawn and the micro-injector (Pneumatic picopump, PV830, World Precision Instruments) was positioned into the guide needle track and the glass pipette tip was inserted into the posterior chamber avoiding the retina. 500 nl of EV solution or control solutions was injected. After completion of the injection, a 10 sec interval was maintained before removing the glass pipette. The glass pipette was removed and ophthalmic antibiotic ointment applied to the injected eye immediately after the intravitreal injection procedure. The animals were then monitored for recovery from anesthesia and then returned to the Weill Cornell Medicine's RARC Facility.

[0162] Evaluation of bio-distribution of intravitreally injected EVs or controls in rodent eyes. The bio-distribution of EV intravitreal injection was analyzed at post injection day 3, week 1, and weeks 2 ($n \geq 3$). Animals were sedated and euthanized in accordance with NIH Animal Welfare guidelines. The eyes were enucleated and placed in 5 ml of 4% formalin in 1X TBS for 16-hr at 4°C and then immersed in 5 ml of 0.5 M sucrose diluted in TBS for 12 h at 4°C. The tissues were mounted in OCT Compound (Tissue-Tek), frozen in a dry-ice/ethanol bath in a Cryomold (Tissue-Tek), immediately serial sectioned from 5 to 40 μm with a cryostat (Leica 3050 S, Leica) and mounted on SuperFrost Plus glass slides (Thermo Fisher Scientific).

Specimens were counterstained with 1 ml of Hoechst stain for 15 min at room temperature. The

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slides were rinsed in 5 ml of TBS (pH 7.4) for 5 min at room temperature. Wash steps were repeated twice. 300 μ l of mounting media was then added and a cover-slip (VWR International LLC) was placed. Slides were imaged with wide field fluorescent microscopy for BSA-fluorescein or bright-field microscopy for H and E stained samples. Unprocessed specimen or mounted slides were stored at -80°C.

[0163] Aqueous EV isolation. Aqueous humor was collected by paracentesis. Briefly, an 18-gauge needle was inserted in the cornea approximately 2 mm anterior to the limbus and then 250 μ L of fluid was removed into a 1 ml syringe. The fluid was immediately transferred to a 1.5 ml siliconized microfuge tube and samples placed on ice. EVs were isolated as described for vitreous EVs.

[0164] Statistical analyses. Graph visualization, calculations were performed using Excel (version 2011, Microsoft). All experiments, unless otherwise stated, were performed with n of ≥ 3 . For nanoparticle tracking analysis particle size, concentration, and distribution was calculated using Stokes- Einstein equation. All error bars are standard deviation and p values < 0.05 for all studies.

Example 1 - Extracellular Vesicles (EV) Escape From Formalin-fixed Bovine Vitreous Tissues and are Retained with 1-ethyl-3-(3-dimethylaminopropyl) Carbodiimide (EDC)-formalin Fixation

[0165] The studies described herein focused on optimizing tissue fixation to retain EVs in the extracellular space. To preserve the histological and morphological structures of tissues, conventional fixation methods employ 10% formalin to create protein-protein crosslinks. The fixation process generally involves processing steps or incubations at or above room temperature; however, elevated temperatures are known to revert formalin protein-protein crosslinks (Shi et al., "Antigen Retrieval in Formalin-fixed, Paraffin-embedded Tissues: An Enhancement Method for Immunohistochemical Staining Based on Microwave Oven Heating of Tissue Sections," *J Histochem Cytochem* 39:741-748 (1991); Ikeda et al., "Extraction and Analysis of Diagnostically Useful Proteins From Formalin-fixed, Paraffin-embedded Tissue Sections," *J Histochem Cytochem* 46:397-403 (1998), which are hereby incorporated by reference in their entirety) and RNA-protein crosslinks (Pena et al., "miRNA In Situ Hybridization in Formaldehyde and EDC-fixed Tissues," *Nat Methods* 6:139-141 (2009), which is hereby incorporated by reference in its entirety). It was hypothesized that the nanometer-sized EVs are lost from formalin-fixed tissue specimens during wash steps at or above room temperature, as shown in a schematic diagram in FIG. 1A. To examine the extent of EV loss from formalin-fixed tissues, formalin-fixed bovine vitreous tissue was immersed in wash buffer at 37°C for various time points and then the supernatant was collected. The ultrastructural

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content of the supernatant was imaged using transmission electron microscopy (TEM) and it was found that a substantial number of EVs were present in the wash buffer and had leaked from the formalin-fixed tissue (FIGs. 1B-1C), as early as 30 minutes. Exposure to temperatures above 4°C also resulted in RNA escape (Pena et al., “miRNA In Situ Hybridization in Formaldehyde and EDC-fixed Tissues,” *Nat Methods* 6:139-141 (2009), which is hereby incorporated by reference in its entirety), and likely protein escape. To permanently retain these nanometer-sized EVs within the tissue and surrounding extracellular space, an additional fixation step was added, in which the water-soluble carbodiimide, EDC, creates a non-reversible crosslink between positively charged amino group side chains and carboxyl groups of EV proteins. Thus, two-step fixation was conducted that involves first fixing samples in formalin and then subsequent cross-linking with EDC. After EDC-formalin fixation, vitreous tissues were placed in wash buffer at various temperatures and the supernatant was imaged with TEM (FIG. 1D). EVs were not detected in the supernatant (FIG. 1E). Particulate matter was observed in the EDC-formalin supernatant, as well as the wash buffer control (FIG. 1F); hence EVs did not escape the EDC-formalin-fixed tissue. To quantitate EV loss from vitreous tissue, Western blotting was used to detect a known exosome marker TSG-101. The supernatant of formalin-fixed vitreous showed significant amount of TSG-101 signal in the supernatant (FIG. 1G). These data suggest that formalin fixed tissues lose a substantial amount of EVs to the wash buffer.

Example 2 - EDC-formalin Fixation of Bovine Vitreous Retains EVs Imaged by Multifocal Microscopy (MPM), When Compared to Formalin Fixation Alone

[0166] The goal was to visualize the structural relationship of EVs in the extracellular space of normal vitreous tissue (FIG. 2A), therefore conventional fixation of bovine vitreous (formalin alone) was compared to EDC-formalin, and then an attempt was made to visualize EVs *in situ*. EVs are known to contain proteins; thus, total protein was labeled in whole mounted samples and then imaged with multiphoton microscopy (FIGs. 2B-2D). To label proteins, a cell permeable fluorescent dye, carboxyfluorescein succinimidyl ester (CFSE) (Bronner-Fraser, M., “Alterations in Neural Crest Migration by a Monoclonal Antibody That Affects Cell Adhesion,” *J Cell Biol* 101:610-617 (1985), which is hereby incorporated by reference in its entirety), was used which covalently links to amines. It was found that formalin-fixed tissues showed positive protein signal near or within the vitreous cells but showed no evidence of extracellular protein signal (FIGs. 2A-2B, n = 4). These data suggested that EVs were either not present in vitreous tissue or were lost during processing of formalin-fixed vitreous specimen. In contrast, EDC-formalin fixed samples showed robust signals for protein in the extracellular matrix consistent in size and shape with EVs (FIGs. 2C-2D). Moreover, EDC-formalin fixed tissues stained with

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CFSE consistently illuminated significantly more EVs (120 fold), when compared to formalin alone (FIG. 2E, $p < 0.05$).

[0167] Bovine EVs imaged by MPM were pleomorphic in size, ranging from approximately 200 to 6000 nm in size, with mean diameter 1513.0 nm (standard error 708.8 nm), and modal size of 800-1400 nm (FIG. 2F). The lower limit of resolution of the multiphoton microscope limited ability to resolve EVs smaller than 200 nm.

Example 3 - Fixation of Bovine Vitreous With EDC-formalin Retains EVs and Extracellular RNA *in situ*

[0168] EVs are also known to contain extracellular RNA (Valadi et al., "Exosome-mediated Transfer of mRNAs and MicroRNAs is a Novel Mechanism of Genetic Exchange Between Cells," *Nat Cell Biol* 9:654-659 (2007), which is hereby incorporated by reference in its entirety), therefore, it was sought to visualize extracellular RNA in vitreous tissues. Bovine vitreous nucleic acids were labeled with propidium iodide (PI), which stains DNA as well as RNA (Suzuki et al., "DNA Staining for Fluorescence and Laser Confocal Microscopy," *J Histochem Cytochem* 45:49-53 (1997), which is hereby incorporated by reference in its entirety), albeit with a lower affinity. Imaging EDC-formalin fixed tissues with confocal microscopy showed signals positive for extracellular RNA and extracellular protein, however no extracellular DNA was detected (FIGs. 3A-3B). Signals for extracellular RNA were found to co-localize within the EV protein signal (FIG. 3A), suggesting that extracellular RNA is within the vesicle. In contrast, fixation with formalin alone resulted in substantially less extracellular RNA and protein signal (FIG. 3C). It was also noted that substantially more RNA was retained within the cytoplasm of vitreous cells in EDC-formalin fixed tissues when compared to conventional fixation. To verify that extracellular PI signal was indeed RNA, EDC-formalin fixed samples were treated with RNase and a significant reduction in extracellular signal was noted (FIGs. 4A-4B). To determine if improvements in EV signals could be observed using a standard fluorescent microscope, images from formalin and EDC-formalin-fixed vitreous samples that were stained with CFSE and PI and then captured with a wide-field fluorescent microscope were compared. The data show EDC-formalin fixed samples demonstrated a strong signal for extracellular protein and RNA, while formalin-fixed specimens failed to show extracellular protein signal (FIGs. 5A-5B). Taken together, these data suggest that EDC-formalin fixation is superior to formalin fixation alone for retaining EV proteins and extracellular RNAs in tissues. Moreover, this technique allows one to determine the spatial relationship of EVs within the vitreous tissue *in situ*.

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Example 4 - Bovine and Human Vitreous Humor Contains EVs

[0169] To correlate the findings observed in the micrographs from EDC-formalin fixed tissues with other methods used to visualize EVs, the ultrastructure of vitreous EVs was studied with TEM (Raposo et al., “B Lymphocytes Secrete Antigen-presenting Vesicles,” *J Exp Med* 183:1161-1172 (1996), which is hereby incorporated by reference in its entirety). Bovine vitreous specimens were negatively stained with uranyl acetate and lead citrate, and the images showed a substantial amount of EVs that were pleomorphic in size (FIG. 6A). Next, EVs isolated from bovine vitreous were labeled with CFSE, an electron dense dye that covalently links to protein amines (Raposo et al., “B Lymphocytes Secrete Antigen-presenting Vesicles,” *J Exp Med* 183:1161-1172 (1996), which is hereby incorporated by reference in its entirety), and images showed an abundance of EVs with dense intra-vesicular staining (FIG. 6B). Since EVs are known to contain RNAs (Valadi et al., “Exosome-mediated Transfer of mRNAs and MicroRNAs is a Novel Mechanism of Genetic Exchange Between Cells,” *Nat Cell Biol* 9:654-659 (2007), which is hereby incorporated by reference in its entirety), EVs isolated from bovine vitreous were imaged after staining with an electron dense and nucleic acid selective dye, acridine orange (AO), that showed positive signal within the EVs (FIG. 6C). Staining whole mount bovine vitreous with ethidium bromide, another electron dense nucleic acid stain, also showed positive signal within the EVs (FIG. 6D). To determine the concentration and size distribution of bovine vitreous EVs, nanoparticle-tracking analysis (NTA) (Dragovic et al., “Sizing and Phenotyping of Cellular Vesicles Using Nanoparticle Tracking Analysis,” *Nanomedicine* 7:780-788 (2011), which is hereby incorporated by reference in its entirety) was used, and it was found that the concentration of extracellular vesicles was at least 2.98×10^7 particles per ml (s.e.m $\pm 8.98 \times 10^6$ particles per ml), corresponding to over 2 billion EVs per bovine eye (FIG. 6E). The data show a heterogeneous extracellular vesicle size, with a mean of 212 nm (s.e.m ± 10 nm), mode of 143 nm (s.e.m ± 20.4 nm), peaks at 125 nm and 215 nm, and some extracellular vesicles measuring up to 550 nm (FIG. 6E). EV size measured by NTA differed from EV size observed by multiphoton microscopy, which is likely the result of ultracentrifugation-based isolation methods that removed larger EVs (van der Pol et al., “Recent Developments in the Nomenclature, Presence, Isolation, Detection and Clinical Impact of Extracellular Vesicles,” *J Thromb Haemost* 14:48-56 (2016), which is hereby incorporated by reference in its entirety). To determine the distribution of vitreous EVs in the whole eye, TEM was performed on post-mortem human eyes and demonstrated numerous vitreous EVs in high concentrations near the vitreous base and ciliary body (FIG. 6F-6G). EVs purified from post-mortem human vitreous specimens and stained with AO also revealed size and shape consistent with EVs (FIG. 6H-6I). These data show that the vitreous EVs are indeed present, are abundant

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in number and heterogeneous in size, and positively stain with CFSE and nucleic acid selective dyes.

Example 5 - Immunohistochemistry Staining of EV-specific Protein TGS-101 in Normal Bovine Vitreous

- 5 [0170] To determine if vitreous EVs expressed EV-associated proteins, proteomic analysis was conducted using liquid chromatography mass spectrometry (LC-MS) and bovine vitreous (cleared of cells with low-speed centrifugation) was compared with the EV isolated fraction (n = 6 of bovine vitreous, samples were pooled). The vitreous and EV isolated fraction showed a total of 1686 protein in the combined proteomic inventory, with 682 and 464 proteins enriched in whole vitreous fraction or EV fraction, respectively, and 540 proteins that were similar in abundance for both. A comprehensive listing of the 1779 proteins detected in the EV and whole vitreous fraction is provided in Table 3, *infra*. The listing of Table 3 identifies the proteins by their protein name (column 1) and protein identifier, which includes their UniProtKB Accession number and name. For each protein listed in Table 3, the log₂ difference in protein amount in the EV fraction compared to cell-free vitreous fraction is listed in column 5, which is based on the amount of protein quantified by label free quantification (LFQ) intensity in the EV-enriched fraction (column 3) and in the cell-free vitreous fraction (column 4). Proteins with enrichment in the EV-fraction are denoted as “EV fraction only” (column 5). The proteins total intensity is represented by the iBAQ value (column 6).
- 20 [0171] Further analysis of the proteome data showed that several known EV-associated proteins are enriched in the EV fraction, including TSG-101 (iBAQ value, which represents protein abundance was 2.30 E+05), CD-9 (iBAQ value, 2.80 E+06), HSP 90-β (iBAQ value, 3.00E+08), and annexin II protein (iBAQ value, 9.40 E+05), as shown in **Table 1**.

Table 1. Exosome marker proteins present and enriched in bovine vitreous EVs when compared to cell-free vitreous.

Protein Name	Vesicular body fraction compared to vitreous Log2 ratio	iBAQ Vitreous	iBAQ vesicular body	**Refs.
CD9 antigen	Vesicular body fraction	2.80E+06	2.80E+06	1-5
Annexin II	Vesicular body fraction	9.40E+05	9.40E+05	2, 3, 5
*TSG101 protein	Vesicular body fraction	2.30E+05	2.30E+05	3, 5
†HSP 90-beta	1.755888	3.00E+08	3.00E+08	3, 5
†HSP 90-alpha	0.7320843	1.20E+09	1.20E+09	3, 5
Pyruvate kinase	0.8703709	1.30E+09	1.30E+09	5
L-lactate dehydrogenase A chain	1.020498	1.20E+09	1.20E+09	5

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Elongation factor 1-alpha 1	2.292728	7.20E+07	7.20E+07	2, 5
Clathrin heavy chain 1	3.026251	8.60E+06	8.60E+06	5
Alpha-enolase	0.4244232	5.20E+09	5.20E+09	5

*TSG101 = Tumor Susceptibility Gene 101; [†]HSP = Heat shock protein

****References for Table 1 which are hereby incorporated by reference in their entirety:**

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 2. Thery, C. et al. *J Cell Biol* **147**, 599-610 (1999).
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- 10 **[0172]** The analysis also showed that several eye specific proteins are also enriched in EV as compared to the cell free vitreous fraction. These eye specific proteins are listed in **Table 2** below.

Table 2: Eye specific proteins enriched in bovine vitreous EVs

Protein Name	Vesicular body fraction compared to vitreous	LFQ intensity vitreous/exosome	iBaQ	References
Retinaldehyde-binding protein 1	2.08453	32.20889	1.50E+09	1-3
Fibrillin-1	2.192545	32.26072	1.20E+08	4-6
Opticin	2.649029	31.54315	1.10E+09	7-8
Arrestin-C	2.369839	26.13197	1.40E+07	9
Retinol-binding protein 3	2.399818	36.24624	5.40E+09	10-11
Phakinin	Vesicular body fraction only	22.0284	1.60E+05	12-13
11-cis retinol dehydrogenase	Vesicular body fraction only	24.73975	1.60E+06	14-15
Fibulin 5	Vesicular body fraction only	25.49948	5.00E+06	16
RPE-retinal G-protein coupled receptor	Vesicular body fraction only	26.91809	1.80E+07	17-18
Retinoid isomerohydrolase (RPE65)	Vesicular body fraction only	23.69232	6.20E+05	19-20
Rhodopsin	Vesicular body fraction only	23.91497	2.30E+06	21-23

References for Table 2, which are hereby incorporated by reference in their entirety

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[0173] To confirm that protein signals observed in the EDC-formalin-fixed vitreous were indeed EVs, immunohistochemistry (IHC) was conducted to visualize *in situ* distribution of a known exosome protein, TSG-101. EDC-formalin fixation of tissues was incompatible with IHC staining. Moreover, TSG-101 signal was not reliably detectable in formalin-fixed tissues when conducting experiments at room temperature, presumably because EVs were lost to the wash buffer. However, reversal of formalin crosslinks are known to be temperature dependent (Ikeda et al., "Extraction and Analysis of Diagnostically Useful Proteins From Formalin-fixed, Paraffin-embedded Tissue Sections," *J Histochem Cytochem* 46:397-403 (1998), which is hereby incorporated by reference in its entirety) and reversion occurs at a slower rate at colder temperatures. Therefore, IHC was performed on formalin-fixed bovine vitreous specimens at 4°C for all processing steps, except for imaging. IHC showed an abundant amount of punctate TSG-101-positive signal in the extracellular space (FIG. 7A), consistent with the EV spatial distribution in EDC-formalin-fixed tissues stained with CFSE (FIG. 2C). Specificity controls showed no extracellular signal (FIG. 7B). It was found that TSG-101 was 136-fold more likely to be found in the vitreous extracellular space than within vitreous cell bodies (FIG. 7C). Formalin-fixed IHC samples were also co-stained for RNA using PI, but extracellular RNA was unable to be detected (FIG. 7D), further suggesting that EDC-formalin fixation is necessary for retaining EV-associated RNAs. These data demonstrate that vitreous EVs contain EV protein markers and can be imaged with IHC under low temperature conditions.

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Example 6 - Vitreous EVs Transfer Endogenous RNA Recombinant Bovine Serum Albumin (BSA), and Green Fluorescent Protein (GFP) into Cultured Cells

[0174] Whether vitreous EVs have biological activity similar to other EVs, which are known to transfer their RNA and protein cargo into target cells, was investigated. Early studies showed that EVs carry mRNAs and microRNAs into cells *in vitro* (Valadi et al., “Exosome-mediated Transfer of mRNAs and MicroRNAs is a Novel Mechanism of Genetic Exchange Between Cells,” *Nat Cell Biol* 9:654-659 (2007); Skog et al., “Glioblastoma Microvesicles Transport RNA and Proteins That Promote Tumour Growth and Provide Diagnostic Biomarkers” *Nat Cell Biol* 10:1470-1476 (2008), which are hereby incorporated by reference in its entirety).

Therefore, bovine and post-mortem human vitreous EVs’ capacity to transfer their endogenous RNA into cultured cells was tested. Bovine or human vitreous EV RNA were labeled with AO fluorescent dye, the EV fraction was purified (FIGs. 8A-8B), and retinal pigment epithelial cells (ARPE-19) were exposed to a bolus of the labeled EVs. For bovine EV-RNA, transfection rate of up to 96.2% $\pm$ 0.9% was observed at 48 hours in cultured ARPE-19 cells (FIGs. 9A-9C), which was significantly more than controls. Human embryonic kidney cells were also transfected successfully (FIG. 9D-9F). Isolated post-mortem human vitreous EVs were also capable of transferring labelled EV-RNA at 96% $\pm$ 3.8% in ARPE-19 cells at 24 hours, significantly more when compared to the controls (FIGs. 9G-9I).

[0175] EVs are also known to be a vector capable of delivering recombinant protein to target cells. Therefore, bovine serum albumin (BSA, 66 kD protein) conjugated to fluorophore (fluorescein) was loaded into 3 μ g of bovine vitreous EVs via electroporation at 300 V, the BSA-fluorescein-loaded EVs were repurified, and then cultured ARPE-19 cells were treated with the vector. It was observed that cells were transfected at 97.6% $\pm$ 0.85%, 95.3% $\pm$ 2.428%, and 88.9% $\pm$ 1.745% for concentrations of BSA-fluorescein of 3 μ g, 1 μ g, and 0.5 μ g, respectively. The controls, PBS alone or EVs mixed with BSA-fluorescein without electroporation, did not result in transfection of ARPE-19 cells (FIGs. 10A-10C), and these groups were statistically different than test groups ($p < 0.05$, $n = 3$). These control data demonstrated that the EV vector was necessary for ARPE-19 cell uptake of BSA-fluorescein. To evaluate whether vitreous EVs are capable of transfecting a functional protein, which must retain its conformational state to fluoresce, recombinant green fluorescent protein (GFP) was loaded into 3 μ g vitreous EVs. The data showed that ARPE-19 cells were transfected at 88.3% $\pm$ 4.2%, 81.4% $\pm$ 4.8%, and 72.9% $\pm$ 3.9% for concentrations of GFP at 0.25 μ g, 0.5 μ g, and 1 μ g, respectively (FIGs. 10D-10F). The controls showed no signal, significantly less than the test groups ($p < 0.05$, $n = 3$). These data show that vitreous EVs are capable of transferring their endogenous RNA as well as exogenous, recombinant protein to cells *in vitro*.

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[0176] Bovine vitreous and aqueous EVs are capable of transferring their endogenous protein and RNA into human cells other than ocular cells, such as skin cells. Bovine vitreous EVs endogenous RNAs and endogenous proteins were labeled and transferred into human skin cells at high efficiency as shown in FIGs. 17A-17L. No transfer was observed under control conditions (FIGs. 17M-17R). These data suggest that vitreous EVs have a broad tropism, and can be used as therapeutic delivery vehicles throughout the body for a broad variety of conditions.

Example 7 - Bovine Vitreous EVs Target the Retina and Deliver Recombinant Protein *In Vivo*

[0177] An attempt was made to validate vitreous EV transfection efficiency *in vivo* and determine the target cells in the eye. A dilute amount of EVs (0.025 µg) that were loaded with 0.5 µg of BSA-fluorescein via electroporation were administered to rodent eyes through intravitreal injection, a common technique used for intraocular delivery. On day 3 post-treatment, EVs show no evidence of crossing into the retina and do not penetrate the inner limiting membrane (FIG. 11A). At three weeks post injection, transfection in cells of multiple retinal cell layers was noted (FIG. 11B-11D). For specificity controls, no evidence of transfection was observed with PBS alone or with EV samples mixed with BSA-fluorescein without electroporation. Together, these data show that the vitreous EVs are biologically active and function as a vector to deliver recombinant proteins *in vivo*. Moreover, vitreous EVs target retinal cells and maintain a sustained transfection for up to 3 weeks.

Example 8 - Bovine Vitreous EVs Target the Cornea, Ciliary Body, and Retina to Deliver Recombinant Protein *In Vivo*

[0178] Bovine EVs loaded by electroporation (300 V) with recombinant bovine serum albumin (BSA) conjugated to fluorescein (BSA-fluorescein) were injected into mice eyes. At 3-week post injection mouse eye sections were examined for BSA-fluorescein delivery, and delivery was observed in cornea from endothelial cells and corneal keratocytes as shown in the photomicrographs of FIG. 12A. Images from the control group of bovine EV mixed with BSA-fluorescein without electroporation (0 V) after 3-week injection show no expression in endothelial cells nor corneal keratocytes, but does show non-specific staining of the corneal epithelium (FIG. 12B). FIG. 12C are representative confocal fluorescent photomicrographs from mouse eyes at 3-week post injection of EVs loaded by electroporation (300 V) with BSA-fluorescein show signal in ciliary body, in the non-pigmented ciliary epithelial cells. The images of FIG. 12D show robust expression of BSA-Fluorescein in the photoreceptors, inner plexiform layer (IPL), retinal pigment epithelial (RPE) cells, and choroid. Nuclei in all tissue sections were

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stained with Hoechst blue, and these are shown in the middle panels of FIGS. 12A-12D. Merged images are shown in the far left panels of FIGS. 12A-12D. The images of FIG. 12E show expression of BSA-fluorescein in the retinal pigment epithelial cells (RPE) and choroid.

Example 9 - Bovine Vitreous Vesicular Bodies Loaded with Fluorescent Labeled siRNAs Transfects into Human Retinal Pigment Epithelial Cells with High Efficiency

[0179] To determine if vitreous vesicular bodies are capable of modification with exogenous small interfering RNAs, an anti-GAPDH siRNA conjugated to cyanine 3 was introduced into vesicular bodies using electroporation. ARPE-19 cells were exposed to anti-GAPDH siRNA-Cy3 loaded into vesicular bodies at various electroporation voltages and it was found that siRNA loaded vesicles transfected the cells at greater efficiency at higher voltages (FIGs. 13A-13C) and less efficiency at lower voltages (FIGs. 13D-13F), and was undetectable without electroporation (FIGs. 13G-13H). FIG. 13I is a graph showing the transfection efficiency by electroporation voltage.

Example 10 - Bovine Ciliary Body Non-pigmented Epithelium Produces Abundant Vesicular Bodies that are Released into Intracellular Spaces

[0180] It was next sought to identify the origin of the ocular fluid vesicular bodies. It was hypothesized the vesicular bodies are produced in the ciliary body since; 1) the vitreous humor collagen fibrils are highest in concentration near the vitreous base, which is behind the lens and juxtaposed to the ciliary body and; 2) The ciliary body is known to have a high surface area and produces the aqueous humor. Therefore, TEM was conducted on tissue sections of bovine ciliary body stained with uranyl acetate and budding vesicles were found in the non-pigmented epithelium into intercellular spaces (FIGs. 14A-14D). Vesicular body budding in the pigmented epithelium was not observed (FIG. 14E). These data suggest that the ciliary body is at least in part, a source of vesicular bodies in the eye.

Example 11 - Bovine Vitreous Vesicular Bodies Deliver Proteins in Human Retinal Pigment Epithelial Cells with High Efficiency at Various Voltages

[0181] As discussed *supra*, bovine vesicular bodies contain a diverse proteome. It was reasoned these vesicles could be loaded with exogenous proteins and used as a tool to deliver proteins to target cells. Therefore, whether vitreous vesicular bodies are capable of modification with exogenous proteins was examined. Bovine serum albumin conjugated to fluorescein (BSA-fluorescein) was introduced into vesicular bodies using electroporation at various voltages and ARPE-19 cells were exposed to the loaded BSA-fluorescein vesicles. It was found that BSA-fluorescein loaded vesicles transfected ARPE-19 cells at a substantially higher efficiency when loaded with protein using 350 V (FIGs. 15A-15C) as compared to when loaded with protein

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using 100 V (FIGs. 15D-15F). No transfection was observed in the absence of electroporation (FIGs. 15G-15H).

Example 12 - Bovine Aqueous Humor Contains Abundant Vesicular Bodies

[0182] The high levels of vitreous vesicular bodies led to the hypothesis that vesicular bodies were also likely located in the anterior chamber of the eye within the aqueous humor. Therefore, the aqueous humor was examined with TEM imaging and a wide distribution of vesicular bodies was found (FIGs. 16A-16C). The vesicular body fraction was isolated and vesicular body size and concentration was determined with nanoparticle tracking analysis, which showed a concentration of at least 1.10×10^8 particles per ml (FIG. 16E, s.e.m $\pm 9.25 \times 10^7$ particles per ml, n=10), and a total of 2.7×10^8 particles per ml per eye (FIG. 16E, n=10), with a mean size of 155 nm (s.e.m ± 27.9 nm) and mode of 88.7 nm (s.e.m ± 34.1) nm. Interestingly, the vesicular body size distribution in the aqueous humor was substantially smaller than vitreous vesicular bodies.

Discussion of Examples

[0183] In summary, conventional formalin fixation based techniques result in escape of a substantial amount of EVs from mammalian tissues, which results in inconsistent or negative visualization of EVs *in situ*. However, EDC-formalin fixation significantly improves retention of EVs in tissues and allows for robust EV imaging *in situ*. This method illuminated a previously unidentified network of EVs in the normal vitreous humor of the eye, a tissue long considered to have few biological functions. Moreover, the data presented herein demonstrates that vitreous EVs can be manipulated as a vector to deliver recombinant proteins and nucleic acids molecules *in vitro* and *in vivo*. In conclusion, this method opens up new possibilities for studying the structure and function of EVs in normal or disease tissues specimens including a wide range of diseases thought to be mediated by EVs such as ophthalmic diseases, neurological disorders, and cancers.

[0184] Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.

Table 3: Proteins expressed in the extracellular vesicles fraction and the cell free vitreous fraction.

Protein Name	Protein IDs	LQF intensity vitreous-EV	LQF intensity total vitreous	EV/ whole vitreous Log2 difference	iBAQ
Superoxide dismutase [Cu-Zn]	sp P00442 SODC_BOVIN;tr F1M NQ4 F1MNQ4_BOVIN	22.602	30.464	-7.862	2.90E+09
Alpha-1-acid glycoprotein	tr Q5GN72 Q5GN72_BOVIN;sp Q3SZR3 A1AG_BOVIN;CON_ Q3SZR3	23.646	31.172	-7.526	2.70E+09
Carbonic anhydrase 2	sp P00921 CAH2_BOVIN	22.362	29.354	-6.992	6.20E+08
Uncharacterized protein	tr F1MIL3 F1MIL3_BOVIN	23.282	30.226	-6.943	4.80E+08
Plasminogen	tr E1B726 E1B726_BOVIN;tr A7 E350 A7E350_BOVIN	23.421	30.323	-6.901	3.60E+08
Beta-crystallin B2	sp P02522 CRBB2_BOVIN	25.394	32.077	-6.682	4.10E+09
Vitamin D-binding protein	tr F1N5M2 F1N5M2_BOVIN;CO N_ENSEMBL:ENSBTAP00000 018229;sp Q3MHN5 VTDB_BOV IN;CON_Q3MHN5	25.276	31.824	-6.548	2.10E+09
Contactin-1	tr F1MVI0 F1MVI0_BOVIN;sp Q2 8106 CNTN1_BOVIN	22.155	28.658	-6.503	1.00E+08
N(G),N(G)- dimethylarginine dimethylaminohydrolase 1	sp P56965 DDAH1_BOVIN	23.851	30.220	-6.369	1.00E+09
Beta-2-glycoprotein 1	sp P17690 APOH_BOVIN;CON_ _P17690	22.838	29.009	-6.171	4.10E+08
Isoform 2 of Prosaposin	sp P26779-2 SAP_BOVIN	24.161	30.297	-6.136	6.40E+08

Beta-crystallin A3	sp P11843 CRBA1_BOVIN;sp P11843-2 CRBA1_BOVIN	23.426	29.561	-6.135	9.70E+08
Triosephosphate isomerase	sp Q5E956 TPIS_BOVIN	25.211	31.324	-6.113	2.20E+09
Aspartate aminotransferase, cytoplasmic	sp P33097 AATC_BOVIN	37.271	31.166	-6.105	1.30E+09
Nucleoside diphosphate kinase A 2	sp P52175 NDKA2_BOVIN;sp P52174 NDKA1_BOVIN;tr G1K1A3 G1K1A3_BOVIN	23.196	29.102	-5.906	7.10E+08
Beta-2-microglobulin	sp P01888 B2MG_BOVIN	22.905	28.765	-5.860	1.00E+09
Fascin	tr Q3MHK9 Q3MHK9_BOVIN	22.848	28.699	-5.851	2.00E+08
Retinol-binding protein 4	sp P18902 RET4_BOVIN;tr G1K122 G1K122_BOVIN	24.465	30.314	-5.849	2.00E+09
Ectonucleotide pyrophosphatase/phosphodiesterase family member 2	sp A1A4K5 ENPP2_BOVIN	24.627	30.447	-5.820	4.40E+08
Uncharacterized protein (Fragment)	tr G5E604 G5E604_BOVIN	24.911	30.127	-5.217	3.20E+09
DKK3 protein	tr A6QL81 A6QL81_BOVIN	26.859	32.061	-5.202	3.20E+09
Phosphatidylethanolamine-binding protein 1	sp P13696 PEBP1_BOVIN	26.793	31.988	-5.195	4.50E+09
12 kDa protein	CON__ENSEMBL:ENSBTAP0000014147;tr G3N2D7 G3N2D7_BOVIN	25.722	30.836	-5.115	4.40E+09
Beta A4 crystallin	tr Q6DTZ8 Q6DTZ8_BOVIN;sp P11842 CRBA4_BOVIN	23.947	29.042	-5.095	8.30E+08
SPARC-like 1 (Hevin)	tr Q3SYW7 Q3SYW7_BOVIN	25.349	30.359	-5.009	5.80E+08

Transferrin	CON_Q0IIK2;CON_Q29443;sp Q29443 TRFE_BOVIN;sp P24627 TRFL_BOVIN	29.333	34.318	-4.986	6.80E+09
Secernin-1	sp P83939 SCRN1_BOVIN	23.902	28.854	-4.952	4.40E+08
Amyloid beta (A4) protein	tr Q08E54 Q08E54_BOVIN	27.302	32.122	-4.820	2.20E+09
Protein HP-20 homolog	sp Q2KIT0 HP20_BOVIN;CON_Q2KIT0	24.398	29.126	-4.728	7.30E+08
Fructose-bisphosphate aldolase	tr A6QLL8 A6QLL8_BOVIN	26.352	30.909	-4.557	1.20E+09
Hemopexin	sp Q3SZV7 HEMO_BOVIN	27.158	31.679	-4.520	2.00E+09
Complement factor H	sp Q28085 CFAH_BOVIN;CON_Q28085;tr E1BFN5 E1BFN5_BOVIN	25.819	30.259	-4.441	2.80E+08
Ubiquitin-60S ribosomal protein L40	sp P63048 RL40_BOVIN;sp P62992 RS27A_BOVIN;tr E1B9K1 E1B9K1_BOVIN;sp P0CG53 UBB_BOVIN;sp P0CH28 UBC_BOVIN	27.095	31.526	-4.430	6.80E+09
Prostaglandin-H2 D-isomerase	sp O02853 PTGDS_BOVIN	27.751	32.132	-4.382	1.20E+10
Uncharacterized protein	tr G3MYY6 G3MYY6_BOVIN	23.753	28.122	-4.369	7.00E+07
Peptidyl-prolyl cis-trans isomerase A	sp P62935 PPIA_BOVIN;tr G3X8B1 G3X8B1_BOVIN;tr G3MZS9 G3MZS9_BOVIN;sp A4FV72 PPIE_BOVIN	25.766	30.133	-4.367	1.60E+09
Uncharacterized protein	tr G8JKW7 G8JKW7_BOVIN	25.222	29.501	-4.279	5.30E+08
Gamma-synuclein	sp Q9NZ50 SYUG_BOVIN	23.391	27.653	-4.262	3.60E+08
Alpha-1-antiproteinase	sp P34955 A1AT_BOVIN;CON_P34955	26.848	31.033	-4.185	1.50E+09

Uncharacterized protein	tr F1N4M7 F1N4M7_BOVIN;CO N_Q32PI4	26.330	30.462	-4.132	5.30E+08
Fructose-bisphosphate aldolase	tr Q3ZBY4 Q3ZBY4_BOVIN;tr A 5PK73 A5PK73_BOVIN;sp Q3T0 S5 ALDOB_BOVIN	27.423	31.539	-4.116	1.90E+09
Serpin A3-7	sp A2I7N3 SPA37_BOVIN;CON_ A2I7N3	26.452	30.558	-4.106	1.20E+09
Insulin-like growth factor-binding protein 6	tr F1MUK3 F1MUK3_BOVIN;sp Q05718 IBP6_BOVIN	24.771	28.773	-4.002	5.70E+08
Uncharacterized protein	tr E1BLM2 E1BLM2_BOVIN	25.797	29.650	-3.852	1.90E+08
Superoxide dismutase [Cu-Zn]	tr A3KLR9 A3KLR9_BOVIN	23.538	27.381	-3.843	2.40E+08
Serpin A3-1	sp Q9TTE1 SPA31_BOVIN; CON_Q9TTE1	28.309	32.074	-3.766	3.60E+09
Protein FAM3C	sp A5PKI3 FAM3C_BOVIN	25.873	29.552	-3.679	8.50E+08
Complement factor B	CON_Q3KUS7;sp P81187 CFAB_BOVIN	27.471	31.129	-3.658	9.10E+08
Fatty acid-binding protein, epidermal	sp P55052 FABP5_BOVIN; tr G3N269 G3N269_BOVIN	25.962	29.509	-3.547	1.30E+09
Uncharacterized protein (Fragment)	tr F1N226 F1N226_BOVIN	28.827	32.351	-3.524	2.40E+09
Leucine-rich alpha-2-glycoprotein 1	tr Q2KIF2 Q2KIF2_BOVIN; CON_Q2KIF2	24.082	27.599	-3.517	1.70E+08
44 kDa protein	CON_ENSEMBL: ENSBTAP00000024466	26.445	29.937	-3.492	8.40E+08
Complement component C7	tr F1N045 F1N045_BOVIN; sp Q29RQ1 CO7_BOVIN;CON_ Q29RQ1	23.540	26.987	-3.447	3.30E+07

Transketolase	tr A7Z014 A7Z014_BOVIN; tr A7E3W4 A7E3W4_BOVIN	27.980	31.413	-3.433	1.60E+09
Apolipoprotein A-IV	tr F1N3Q7 F1N3Q7_BOVIN; sp Q32PJ2 APOA4_BOVIN;CON _Q32PJ2	25.676	29.107	-3.431	3.90E+08
Rho GDP- dissociation inhibitor 1	sp P19803 GDIR1_BOVIN; tr Q58DT6 Q58DT6_BOVIN	23.758	27.156	-3.398	2.30E+08
Uncharacterized protein (Fragment)	tr F1MW33 F1MW33_BOVIN	27.383	30.695	-3.312	5.50E+08
Spondin-1	sp Q9GLX9 SPON1_BOVIN	25.918	29.188	-3.270	2.20E+08
Versican core protein	tr F1MZ85 F1MZ85_BOVIN; sp P81282 CSPG2_BOVIN;tr F1 MZ83 F1MZ83_BOVIN;sp P8128 2- 3 CSPG2_BOVIN;tr F1N6I5 F1N 6I5_BOVIN;sp P81282- 2 CSPG2_BOVIN;tr F1N6I7 F1N 6I7_BOVIN;sp P81282- 4 CSPG2_BOVIN	25.871	29.079	-3.208	6.00E+07
Microtubule-associated protein	tr F1MEW3 F1MEW3_BOVIN	25.105	28.293	-3.189	4.90E+07
Hemoglobin subunit alpha	sp P01966 HBA_BOVIN;CON _P01966;tr F1MMI1 F1MMI1_B OVIN;tr E1BAP8 E1BAP8_BOVI N	28.801	31.916	-3.115	6.30E+09
Pigment epithelium -derived factor	sp Q95121 PEDF_BOVIN; CON_Q95121	29.317	32.298	-2.981	3.20E+09
Metalloproteinase inhibitor 2	tr F1N430 F1N430_BOVIN; sp P16368 TIMP2_BOVIN	24.231	27.203	-2.972	1.90E+08

Metalloproteinase inhibitor 1	sp P20414 TIMP1_BOVIN	24.511	27.440	-2.930	3.20E+08
Malate dehydrogenase, cytoplasmic	sp Q3T145 MDHC_BOVIN	27.167	30.066	-2.899	1.10E+09
Rab GDP dissociation inhibitor alpha	sp P21856 GDIA_BOVIN	27.525	30.403	-2.879	8.10E+08
Isoaspartyl peptidase/L-asparaginase	sp Q32LE5 ASGL1_BOVIN	23.567	26.410	-2.842	9.40E+07
Phosphoprotein enriched in astrocytes 15	tr Q0VCY8 Q0VCY8_BOVIN	24.651	27.444	-2.793	3.60E+08
Isoform 3 of Neurexin-3-beta	sp Q28143-3 NRX3B_BOVIN;	23.933	26.721	-2.788	1.10E+08
	sp Q28143 NRX3B_BOVIN;tr F1MR33 F1MR33_BOVIN;sp Q28143-4 NRX3B_BOVIN;sp Q28143-2 NRX3B_BOVIN;tr G3X794 G3X794_BOVIN;tr F6RU36 F6RU36_BOVIN				
	6_BOVIN				
Glucose-6-phosphate isomerase	sp Q3ZBD7 G6PI_BOVIN;CON_Q3ZBD7	28.915	31.682	-2.768	1.80E+09
Latent-transforming growth factor beta-binding protein 2	tr F1MF86 F1MF86_BOVIN;sp Q28019 LTBP2_BOVIN	25.914	28.631	-2.717	7.90E+07
Cathepsin B	sp P07688 CATB_BOVIN	24.321	27.005	-2.684	1.10E+08
Macrophage migration inhibitory factor	sp P80177 MIF_BOVIN	25.576	28.244	-2.668	8.40E+08
Alpha-crystallin A chain	sp P02470 CRYAA_BOVIN	30.314	32.963	-2.649	1.10E+10
similar to endopin 2B	CON_REFSEQ:XP_001252647	28.386	31.016	-2.630	1.70E+09
Haptoglobin	sp Q2TBU0 HPT_BOVIN;	26.301	28.923	-2.622	2.60E+08
	tr G3X6K8 G3X6K8_BOVIN				

Uncharacterized protein	tr F1MI18 F1MI18_BOVIN;	23.474	26.063	-2.589	1.30E+07
Beta-crystallin S	tr F1MJK3 F1MJK3_BOVIN	24.716	27.285	-2.569	2.60E+08
WAP, Kazal, immunoglobulin, Kunitz and NTR domain-containing protein 2	sp P06504 CRBS_BOVIN				
	tr F1MRR8 F1MRR8_BOVIN; sp Q08E66 WFKN2_BOVIN	26.327	28.853	-2.526	3.30E+08
Histidine triad nucleotide-binding protein 1	sp P62958 HINT1_BOVIN	25.815	28.317	-2.502	7.60E+08
Seizure protein 6 homolog	tr F1N649 F1N649_BOVIN; sp A0JNA2 SEZ6_BOVIN	27.474	29.972	-2.498	4.50E+08
Glutathione S-transferase Mu 1	tr A1A4L7 A1A4L7_BOVIN	24.255	26.706	-2.451	1.00E+08
Beta-crystallin A2	sp P26444 CRBA2_BOVIN	24.556	26.966	-2.411	1.60E+08
Serum albumin	sp P02769 ALBU_BOVIN; CON_P02769	33.997	36.325	-2.329	3.20E+10
Protein CutA	tr F1MT17 F1MT17_BOVIN; tr F1N5T0 F1N5T0_BOVIN;sp P69678 CUTA_BOVIN	25.272	27.584	-2.312	4.70E+08
	tr F1MZ96 F1MZ96_BOVIN; tr F1MH40 F1MH40_BOVIN;CO N_Q05B55	26.892	29.189	-2.297	7.90E+08
Uncharacterized protein	sp Q03247 APOE_BOVIN; CON_Q03247	28.872	31.167	-2.295	2.30E+09
Apolipoprotein E	tr F1MMJ2 F1MMJ2_BOVIN; sp P31836 NCAM1_BOVIN	26.725	28.952	-2.227	2.10E+08
Neural cell adhesion molecule 1 (Fragment)	tr F1MYE4 F1MYE4_BOVIN; sp Q27972 CHAD_BOVIN	25.400	27.601	-2.202	1.90E+08
Chondroadherin	sp Q27972 CHAD_BOVIN				
Protein DJ-1	sp Q5E946 PARK7_BOVIN	27.638	29.820	-2.182	1.00E+09

Nucleoside diphosphate kinase B	sp Q3T0Q4 NDKB_BOVIN; tr F1MPL4 F1MPL4_BOVIN	24.330	26.507	-2.177	1.40E+08
Calbindin	sp P04467 CALB1_BOVIN	25.302	27.430	-2.128	1.70E+08
Protein AMBP	tr F1MMK9 F1MMK9_BOVIN; sp P00978 AMBP_BOVIN;CON_P00978	25.095	27.186	-2.090	1.30E+08
	Uncharacterized protein (Fragment)	29.489	31.555	-2.066	1.30E+09
Protein S100-A1	tr F1MD95 F1MD95_BOVIN; sp P02639 S10A1_BOVIN; tr H9KUV1 H9KUV1_BOVIN	27.359	29.412	-2.053	3.80E+09
	sp P36225-3 MAP4_BOVIN; tr F1MAZ3 F1MAZ3_BOVIN;sp P36225-4 MAP4_BOVIN;tr G3N2G7 G3N2G7_BOVIN;tr F1MAZ1 F1MAZ1_BOVIN;sp P36225-N;sp P36225-2 MAP4_BOVIN;tr F1N0J2 F1N0J2_BOVIN	25.476	27.522	-2.045	4.80E+07
Isoform 3 of Microtubule-associated protein 4	tr E1BBY7 E1BBY7_BOVIN	24.581	26.626	-2.045	3.00E+07
Uncharacterized protein	tr Q17QV0 Q17QV0_BOVIN; sp Q0VCU0 PCBP4_BOVIN	29.413	27.398	2.015	2.00E+08
Poly(RC) binding protein 3	tr E1BNY5 E1BNY5_BOVIN; tr G3N1P4 G3N1P4_BOVIN;tr F6R352 F6R352_BOVIN;tr E1BMQ2 E1BMQ2_BOVIN;tr F1MEH0 F1MEH0_BOVIN	27.656	25.618	2.038	3.30E+07
	Uncharacterized protein	30.598	28.541	2.056	1.30E+08

Retinaldehyde-binding protein 1	sp P10123 IRLBP1_BOVIN	32.209	30.124	2.085	1.50E+09
Cullin-associated NEDD8-dissociated protein 1	sp A7MBJ5 CAND1_BOVIN;tr E1BNE2 E1BNE2_BOVIN;tr G3N157 G3N157_BOVIN	29.951	27.856	2.095	6.30E+07
	tr A6QR61 A6QR61_BOVIN	27.838	25.738	2.099	7.10E+07
	tr E1BHR3 E1BHR3_BOVIN	27.565	25.465	2.100	1.20E+08
	sp Q95114 MFGM_BOVIN;tr F1MXX6 F1MXX6_BOVIN;sp Q95114-2 MFGM_BOVIN;tr G3MYW7 G3MYW7_BOVIN	25.996	23.886	2.109	9.40E+06
Lactadherin					
>P35527 SWISS-PROT:P35527 Tax_Id=9606 Gene_Symbol=KRT9 Keratin, type I cytoskeletal 9	CON_P35527;tr G3X7W8 G3X7W8_BOVIN;CON_Q99456;tr G3MX98 G3MX98_BOVIN	28.971	26.835	2.136	7.20E+07
	sp Q2KIA5 DNM1L_BOVIN	28.918	26.778	2.140	3.60E+07
	tr A2VE41 A2VE41_BOVIN	31.663	29.517	2.146	6.20E+08
EGF-containing fibulin-like extracellular matrix protein 1	tr E1BD68 E1BD68_BOVIN;tr F1MC12 F1MC12_BOVIN;sp O18964 SYNJ1_BOVIN	27.268	25.112	2.156	8.50E+06
Synaptojanin-1	sp Q08DF4 DYN1_BOVIN	29.583	27.425	2.158	5.40E+07
Dynamamin-1	tr F1N4K8 F1N4K8_BOVIN;sp P98133 FBN1_BOVIN;tr F1MTZ4 F1MTZ4_BOVIN	32.261	30.068	2.193	1.20E+08
Fibrillin-1					
SUB1 protein	tr A7YWC6 A7YWC6_BOVIN	26.815	24.615	2.200	9.50E+07

Uncharacterized protein (Fragment)	tr F1MJ70 F1MJ70_BOVIN;tr A6QLJ2 A6QLJ2_BOVIN	29.150	26.935	2.215	7.80E+07
Oxidation resistance protein 1 (Fragment)	tr F1MR28 F1MR28_BOVIN;sp A5PKL1 OXR1_BOVIN;tr Q0IIB3 Q0IIB3_BOVIN	26.788	24.498	2.290	1.10E+07
	sp P68103 EF1A1_BOVIN;tr E1B9F6 E1B9F6_BOVIN;tr G3N0P6 G3N0P6_BOVIN;tr E1BED8 E1BED8_BOVIN;tr E1B7J1 E1B7J1_BOVIN;tr G3N2F0 G3N2F0_BOVIN;tr E1BPF4 E1BPF4_BOVIN	28.821	26.528	2.293	7.20E+07
Elongation factor 1-alpha 1	tr F1MPE5 F1MPE5_BOVIN;tr F1N074 F1N074_BOVIN	27.638	25.338	2.300	9.30E+06
Syntaxin-binding protein 1	tr F6R0H3 F6R0H3_BOVIN	30.466	28.153	2.313	1.90E+08
	tr E1BFV0 E1BFV0_BOVIN	26.789	24.451	2.337	1.10E+07
Eukaryotic initiation factor 4A-II	sp Q3SZ65 IF4A2_BOVIN	30.167	27.826	2.340	1.60E+08
Calcyclin-binding protein	sp Q3T168 CYBP_BOVIN	27.032	24.684	2.348	3.90E+07
Mitogen-activated protein kinase 1	sp P46196 MK01_BOVIN;tr F1MI27 F1MI27_BOVIN;tr F1MNV5 F1MNV5_BOVIN;tr G5E577 G5E577_BOVIN	28.682	26.331	2.350	8.60E+07
	tr F1N5F9 F1N5F9_BOVIN	27.727	25.376	2.351	3.10E+07
Poly(RC) binding protein 2	tr Q3SYT9 Q3SYT9_BOVIN	26.633	24.280	2.352	2.80E+07
Arrestin-C	sp Q9N0H5 ARRC_BOVIN;tr F1MCQ1 F1MCQ1_BOVIN	26.132	23.762	2.370	1.40E+07

V-type proton ATPase subunit B, brain isoform	sp P31408 VATB2_BOVIN;tr F1N688 F1N688_BOVIN;sp P31407 VATB1_BOVIN	27.441	25.051	2.389	2.10E+07
Retinol-binding protein 3	sp P12661 RET3_BOVIN	36.246	33.846	2.400	5.40E+09
Uncharacterized protein (Fragment)	tr F1MKZ3 F1MKZ3_BOVIN	29.234	26.796	2.438	2.70E+07
Isoform Beta of V-type proton ATPase subunit H	sp O46563-2 VATH_BOVIN;sp O46563 VATH_BOVIN;tr F1MZL6 F1MZL6_BOVIN;tr F1MZL8 F1MZL8_BOVIN	25.964	23.504	2.460	7.70E+06
>P15636 SWISS-PROT:P15636 Protease I precursor Lysyl endopeptidase Achromobacter lyticus.	CON_P15636	33.776	31.310	2.466	1.60E+09
Histone H3.3	sp Q5E9F8 H33_BOVIN;tr G3MYD7 G3MYD7_BOVIN;sp A5PK61 H3C_BOVIN;tr G3N2P2 G3N2P2_BOVIN;tr E1BGN3 E1BGN3_BOVIN;sp P84227 H32_BOVIN;sp P68432 H31_BOVIN;sp Q3SZB8 H3CL_BOVIN	27.521	25.025	2.496	8.70E+07
ADP-ribosylation factor 4	sp Q3SZF2 ARF4_BOVIN	27.440	24.914	2.525	4.80E+07
Elongation factor 1-alpha 2	sp Q32PH8 EF1A2_BOVIN	31.216	28.670	2.546	3.40E+08

Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase	tr Q2HJ26 Q2HJ26_BOVIN;tr F1MN04 F1MN04_BOVIN	27.307	24.756	2.551	2.70E+07
ADP-ribosylation factor 3	sp Q5E9I6 ARF3_BOVIN	30.361	27.800	2.561	3.60E+08
Uncharacterized protein	tr F2Z4C1 F2Z4C1_BOVIN;sp Q3ZCJ7 TBA1C_BOVIN;tr F1MNF8 F1MNF8_BOVIN;tr F2Z4K0 F2Z4K0_BOVIN;tr F6RP72 F6RP72_BOVIN;sp Q32KN8 TBA3_BOVIN	29.832	27.246	2.586	1.70E+08
	tr F1MVT7 F1MVT7_BOVIN	27.080	24.460	2.620	1.50E+07
	sp P58874 OPT_BOVIN	31.543	28.894	2.649	1.10E+09
	tr E1BMU2 E1BMU2_BOVIN	27.283	24.606	2.677	2.90E+07
Uncharacterized protein	tr F1MQ37 F1MQ37_BOVIN	25.781	23.103	2.678	2.00E+06
SERPIND1 protein	tr A6QPP2 A6QPP2_BOVIN;CO N_ENSEMBL:ENSBTAP00000018574	30.392	27.707	2.685	1.60E+08
Uncharacterized protein (Fragment)	tr E1BKM4 E1BKM4_BOVIN	27.286	24.592	2.695	9.60E+06
Glyceraldehyde-3-phosphate dehydrogenase	sp P10096 G3P_BOVIN;sp Q2KJE5 G3PT_BOVIN;tr E1BH84 E1BH84_BOVIN	34.591	31.867	2.724	4.90E+09
cAMP-dependent protein kinase catalytic subunit beta	sp P05131 KAPCB_BOVIN;tr E1BEN2 E1BEN2_BOVIN;sp P05131-2 KAPCB_BOVIN	26.843	24.113	2.730	1.60E+07

T-complex protein 1 subunit gamma	sp Q3T0K2 TCPG_BOVIN;tr F1N5P4_BOVIN	25.906	23.121	2.784	5.80E+06
Spliceosome RNA helicase DDX39B	sp Q3T147 DX39B_BOVIN;tr Q5E970 Q5E970_BOVIN	29.131	26.341	2.790	8.70E+07
Acid ceramidase	sp Q17QB3 ASAH1_BOVIN	25.522	22.707	2.815	6.30E+06
GSK3A protein	tr A6QLB8 A6QLB8_BOVIN	26.003	23.144	2.859	1.00E+07
APPL1 protein	tr A5PKI0 A5PKI0_BOVIN;tr Q1RMW4 Q1RMW4_BOVIN	25.936	23.025	2.910	3.70E+06
T-complex protein 1 subunit eta (Fragment)	tr F1MWR8 F1MWR8_BOVIN;sp Q2NKZ1 TCPH_BOVIN	25.932	22.997	2.936	5.20E+06
Leukocyte cell-derived chemotaxin 1	sp P17404 LECT1_BOVIN	29.516	26.574	2.942	1.20E+08
Clathrin heavy chain 1	sp P49951 CLH1_BOVIN;tr F1MPU0 F1MPU0_BOVIN	28.006	24.980	3.026	8.60E+06
RAB14 protein	tr Q3ZBG1 Q3ZBG1_BOVIN	26.733	23.664	3.068	1.70E+07
Uncharacterized protein (Fragment)	tr E1BFD5 E1BFD5_BOVIN;tr F1N3S4 F1N3S4_BOVIN	26.362	23.255	3.107	4.00E+06
Uncharacterized protein	tr F1MQI1 F1MQI1_BOVIN	26.483	23.371	3.112	1.80E+06
N-acylneuraminate cytidyltransferase	sp Q3SZM5 NEUA_BOVIN;tr E1B9W3 E1B9W3_BOVIN	28.044	24.454	3.590	2.50E+07
Uncharacterized protein (Fragment)	tr F1N1S8 F1N1S8_BOVIN	26.724	23.077	3.647	9.30E+06
Isoform IB of Synapsin-1	sp P17599-2 SYN1_BOVIN;sp P17599 SYN1_BOVIN	27.294	23.644	3.649	8.80E+06
Fatty acid synthase	tr F1N647 F1N647_BOVIN	29.253	25.490	3.763	1.10E+07
Uncharacterized protein	tr E1BE98 E1BE98_BOVIN	27.384	23.552	3.831	6.00E+06

Exportin-2	tr F1MWN1 F1MWN1_BOVIN;sp A5D785 XPO2_BOVIN;tr Q58DL4 Q58DL4_BOVIN	26.849	22.975	3.874	4.40E+06
T-complex protein 1 subunit beta	sp Q3ZBH0 TCPB_BOVIN	26.928	22.900	4.028	7.10E+06
MGC139254 protein	tr A5D7E1 A5D7E1_BOVIN;tr E1BJV0 E1BJV0_BOVIN	27.409	22.675	4.734	9.90E+06
Platelet-activating factor acetylhydrolase	sp Q28017 PAFA_BOVIN;tr Q1RML9 Q1RML9_BOVIN	28.048	22.764	5.284	1.80E+07
Ras-related protein Rab-21	sp Q17R06 RAB21_BOVIN	20.130	NaN	EV fraction only	8.80E+04
Chromosome 14 open reading frame 166 ortholog	tr Q3T0S7 Q3T0S7_BOVIN	20.158	NaN	EV fraction only	7.30E+04
Uncharacterized protein	tr F1MEP1 F1MEP1_BOVIN;sp Q1JP73 CI064_BOVIN	20.318	NaN	EV fraction only	5.30E+06
Copine I	tr Q08DB4 Q08DB4_BOVIN	20.398	NaN	EV fraction only	6.00E+04
Uncharacterized protein	tr F1MVC0 F1MVC0_BOVIN	20.517	NaN	EV fraction only	1.50E+04
Uncharacterized protein	tr G3MZK0 G3MZK0_BOVIN	20.538	NaN	EV fraction only	9.50E+04
Basal cell adhesion molecule	sp Q9MZ08 BCAM_BOVIN	20.565	NaN	EV fraction only	5.30E+04
Ubiquitin carboxyl-terminal hydrolase	tr Q0IIM6 Q0IIM6_BOVIN	20.718	NaN	EV fraction only	3.30E+04
Uncharacterized protein	tr G3N3D4 G3N3D4_BOVIN	20.722	NaN	EV fraction only	1.20E+05
Uncharacterized protein	tr E1BFB0 E1BFB0_BOVIN	20.766	NaN	EV fraction only	1.20E+04

Uncharacterized protein (Fragment)	tr F1MLQ5 F1MLQ5_BOVIN	20.857	NaN	EV fraction only	5.40E+04
ATP-dependent RNA helicase DDX19A	sp Q3ZBV2 DD19A_BOVIN;tr F1MUT6 F1MUT6_BOVIN;tr Q58DE5 Q58DE5_BOVIN	20.898	NaN	EV fraction only	7.00E+04
				EV fraction only	
60S ribosomal protein L24	sp Q86211 RL24_BOVIN	20.938	NaN	EV fraction only	2.50E+05
Phosphomannomutase 2	sp Q3SZJ9 PMM2_BOVIN	20.968	NaN	EV fraction only	1.30E+05
Eukaryotic translation initiation factor 2 subunit 2	sp Q5E9D0 IF2B_BOVIN	21.116	NaN	EV fraction only	1.10E+05
26S proteasome non-ATPase regulatory subunit 6	tr F1MXE4 F1MXE4_BOVIN;sp Q3T0B2 PSMD6_BOVIN	21.119	NaN	EV fraction only	9.10E+04
				EV fraction only	
Uncharacterized protein	tr F1MJJ9 F1MJJ9_BOVIN	21.148	NaN	EV fraction only	7.50E+04
Uncharacterized protein	tr F1MLE8 F1MLE8_BOVIN	21.156	NaN	EV fraction only	1.80E+05
RFK protein (Fragment)	tr Q3SZP4 Q3SZP4_BOVIN	21.203	NaN	EV fraction only	2.20E+05
40S ribosomal protein S18	sp Q3T0R1 RS18_BOVIN	21.227	NaN	EV fraction only	2.70E+05
	tr F1MK51 F1MK51_BOVIN;tr H7BWW0 H7BWW0_BOVIN	21.237	NaN	EV fraction only	1.90E+05
Nucleosome assembly protein 1-like 4	tr F1N7X3 F1N7X3_BOVIN;sp Q2TA40 NP1L4_BOVIN	21.250	NaN	EV fraction only	1.60E+05
Vacuolar protein sorting-associated protein 29 (Fragment)	tr G3X6P5 G3X6P5_BOVIN;sp Q3T0M0 VPS29_BOVIN	21.252	NaN	EV fraction only	2.50E+05

60S ribosomal protein L6	tr G3N2R1 G3N2R1_BOVIN;sp Q58DQ3 RL6_BOVIN	21.302	NaN	EV fraction only	2.20E+05
Golgi phosphoprotein 3-like	sp A6H7F6 GLP3L_BOVIN;tr Q1RMW9 Q1RMW9_BOVIN	21.370	NaN	EV fraction only	1.60E+05
Hemoglobin fetal subunit beta	sp P02081 HBBF_BOVIN;CON_Q3SX09;tr G3MZ21 G3MZ21_BOVIN;tr G3N1Y3 G3N1Y3_BOVIN;tr E1BEL8 E1BEL8_BOVIN;sp P06643 HBE4_BOVIN;sp P06642 HBE2_BOVIN	21.380	NaN	EV fraction only	2.50E+05
Keratin, type II cytoskeletal 5	tr M0QVZ6 M0QVZ6_BOVIN;CON_Q922U2;tr A5D7M6 A5D7M6_BOVIN;sp Q5XQN5 K2C5_BOVIN;CON_Q5XQN5;sp Q08D91 K2C75_BOVIN;CON_Q8BGZ7;CON_P50446;tr G3MXL3 G3MXL3_BOVIN	21.381	NaN	EV fraction only	8.80E+04
GALE protein	tr Q3T105 Q3T105_BOVIN	21.392	NaN	EV fraction only	1.60E+05
Uncharacterized protein	tr E1BAB9 E1BAB9_BOVIN	21.399	NaN	EV fraction only	4.00E+05
Dystrobrevin	tr E1BMA8 E1BMA8_BOVIN;tr E1BJB8 E1BJB8_BOVIN	21.405	NaN	EV fraction only	6.20E+04
Uncharacterized protein	tr G3X696 G3X696_BOVIN	21.418	NaN	EV fraction only	4.40E+04
Dehydrogenase/reductase SDR family member 11	sp Q3ZBV9 DHR11_BOVIN	21.497	NaN	EV fraction only	2.00E+05
Uncharacterized protein	tr F1N5V9 F1N5V9_BOVIN	21.514	NaN	EV fraction only	5.00E+05

IST1 homolog	tr F1MXJ5 F1MXJ5_BOVIN;sp Q3ZBV1 IST1_BOVIN	21.557	NaN	EV fraction only	2.80E+05
Methylthioribose-1-phosphate isomerase	sp Q2NL31 MTNA_BOVIN	21.568	NaN	EV fraction only	1.60E+05
ADP-ribosylation factor 2	sp P84081 ARF2_BOVIN	21.594	NaN	EV fraction only	3.20E+05
PPCS protein	tr A6QPS1 A6QPS1_BOVIN	21.596	NaN	EV fraction only	3.20E+05
Uncharacterized protein (Fragment)	tr F1MJZ4 F1MJZ4_BOVIN	21.601	NaN	EV fraction only	9.60E+04
Eukaryotic translation initiation factor 3 subunit K	sp Q3T0V3 EIF3K_BOVIN	21.663	NaN	EV fraction only	3.00E+05
Uncharacterized protein	tr F6QK60 F6QK60_BOVIN	21.715	NaN	EV fraction only	1.50E+05
DnaJ homolog subfamily A member 2	sp Q2HJ94 DNJA2_BOVIN	21.741	NaN	EV fraction only	1.60E+05
Uncharacterized protein (Fragment)	tr F1N605 F1N605_BOVIN	21.751	NaN	EV fraction only	1.60E+05
DMD protein	tr A8WFL6 A8WFL6_BOVIN	21.753	NaN	EV fraction only	1.30E+05
Uncharacterized protein	tr F1ML33 F1ML33_BOVIN;sp Q08DM5 CS012_BOVIN	21.757	NaN	EV fraction only	5.10E+05
Protein phosphatase 2, regulatory subunit B, alpha isoform	tr A2VDZ0 A2VDZ0_BOVIN	21.839	NaN	EV fraction only	1.60E+05
ADP-ribosylation factor	tr A6QR32 A6QR32_BOVIN;sp A1L520 ARFG2_BOVIN	21.878	NaN	EV fraction only	1.40E+05
Uncharacterized protein (Fragment)	tr E1BMF5 E1BMF5_BOVIN;tr A6QR52 A6QR52_BOVIN	21.901	NaN	EV fraction only	1.20E+05

Isoform 2 of Cell division control protein 42 homolog	sp Q2KJ93-2 CDC42_BOVIN;tr F1N5L2 F1N5L2_BOVIN	21.940	NaN	EV fraction only	4.50E+05
Isoform B of Phosphate carrier protein, mitochondrial	sp P12234-2 MPCP_BOVIN;sp P12234 MP	21.968	NaN	EV fraction only	2.30E+05
Uncharacterized protein (Fragment)	tr F1MZX3 F1MZX3_BOVIN	21.994	NaN	EV fraction only	1.90E+06
Uncharacterized protein (Fragment)	tr E1BMW2 E1BMW2_BOVIN;tr G5E675 G5E675_BOVIN;sp Q0VCK5 AP2A2_BOVIN	21.998	NaN	EV fraction only	1.30E+06
LRRC47 protein	tr A6QR01 A6QR01_BOVIN	22.020	NaN	EV fraction only	1.50E+05
Phakinin	sp Q28177 BFSP2_BOVIN;tr F1MR92 F1MR92_BOVIN	22.028	NaN	EV fraction only	1.60E+05
Uncharacterized protein	tr G3N2H3 G3N2H3_BOVIN;tr F1MIK1 F1MIK1_BOVIN;tr E1BLC8 E1BLC8_BOVIN	22.037	NaN	EV fraction only	5.80E+04
Protein kinase C delta type	tr F1MZX0 F1MZX0_BOVIN;tr E1BMG4 E1BMG4_BOVIN	22.040	NaN	EV fraction only	1.10E+05
TSG101 protein	tr A3KN51 A3KN51_BOVIN	22.053	NaN	EV fraction only	2.30E+05
26S protease regulatory subunit 10B	tr F1MLV1 F1MLV1_BOVIN;sp Q2KIW6 PRS10_BOVIN	22.056	NaN	EV fraction only	2.10E+05
Uncharacterized protein	tr G5E6N8 G5E6N8_BOVIN	22.072	NaN	EV fraction only	1.90E+05
Uncharacterized protein	tr E1BKE3 E1BKE3_BOVIN;tr E1B999 E1B999_BOVIN;tr F1N020 F1N020_BOVIN	22.082	NaN	EV fraction only	3.80E+06

Uncharacterized protein	tr E1BIE5 E1BIE5_BOVIN	22.102	NaN	EV fraction only	2.50E+05
LGI4 protein	tr A6QLD0 A6QLD0_BOVIN	22.115	NaN	EV fraction only	1.60E+05
Very-long-chain enoyl-CoA reductase	sp Q3ZCD7 TECR_BOVIN	22.143	NaN	EV fraction only	3.60E+05
Uncharacterized protein	tr E1BLS8 E1BLS8_BOVIN	22.162	NaN	EV fraction only	9.20E+04
Ubiquitin-like modifier activating enzyme 3	tr Q0P5I7 Q0P5I7_BOVIN	22.171	NaN	EV fraction only	2.10E+05
Phosphorylase (Fragment)	tr F1MU24 F1MU24_BOVIN	22.185	NaN	EV fraction only	1.20E+05
Uncharacterized protein (Fragment)	tr F1MD34 F1MD34_BOVIN	22.218	NaN	EV fraction only	1.40E+05
Serine threonine kinase 39 (STE20/SPS1 homolog, yeast)	tr Q32LA8 Q32LA8_BOVIN	22.244	NaN	EV fraction only	5.50E+05
Uncharacterized protein	tr E1BM26 E1BM26_BOVIN	22.261	NaN	EV fraction only	3.90E+05
Similar to Immunoglobulin lambda-like polypeptide 1	CON_Q1RMN8	22.314	NaN	EV fraction only	5.80E+05
Sulfurtransferase	tr Q3MHG3 Q3MHG3_BOVIN	22.325	NaN	EV fraction only	3.50E+05
EH-domain containing 2	tr Q2KJ47 Q2KJ47_BOVIN	22.328	NaN	EV fraction only	1.80E+05
Selenocysteine lyase	sp A2VDS1 SCLY_BOVIN	22.376	NaN	EV fraction only	2.40E+05

Oxysterol-binding protein	tr E1BLV1 E1BLV1_BOVIN;tr Q58DI6 Q58DI6_BOVIN;tr F1MYP7 F1MYP7_BOVIN;tr E1BL67 E1BL67_BOVIN	22.404	NaN	EV fraction only	1.10E+05
Uncharacterized protein (Fragment)	tr F1MCY0 F1MCY0_BOVIN	22.444	NaN	EV fraction only	1.90E+05
Kinesin-associated protein 3	tr Q3MHI2 Q3MHI2_BOVIN	22.496	NaN	EV fraction only	1.70E+05
Uncharacterized protein	tr F1MCK4 F1MCK4_BOVIN	22.500	NaN	EV fraction only	4.20E+05
Calcium/calmodulin-dependent protein kinase type II subunit beta	sp Q3MHJ9 KCC2B_BOVIN;tr F1MG86 F1MG86_BOVIN;tr F1MVF1 F1MVF1_BOVIN	22.509	NaN	EV fraction only	2.20E+05
Protein kinase C and casein kinase substrate in neurons 2				EV fraction only	2.50E+05
Alpha-soluble NSF attachment protein	tr Q1RMR9 Q1RMR9_BOVIN tr A5D7S0 A5D7S0_BOVIN;sp P81125 SNAA_BOVIN	22.568 22.585	NaN NaN	EV fraction only EV fraction only	3.50E+05
Uncharacterized protein	tr F1N2Y2 F1N2Y2_BOVIN	22.590	NaN	EV fraction only	8.50E+04
Protein kinase C epsilon type	tr F1MDC9 F1MDC9_BOVIN;tr F1MY82 F1MY82_BOVIN	22.595	NaN	EV fraction only	1.50E+05
Oxysterol-binding protein (Fragment)	tr E1BPW1 E1BPW1_BOVIN	22.596	NaN	EV fraction only	1.70E+05
Uncharacterized protein	tr F1N6X2 F1N6X2_BOVIN	22.606	NaN	EV fraction only	2.50E+05
Coronin-1A	sp Q92176 COR1A_BOVIN	22.614	NaN	EV fraction only	3.40E+05

60S ribosomal protein L3	sp P39872 RL3_BOVIN	22.616	NaN	EV fraction only	3.40E+05
Transportin-1 (Fragment)	tr F1MMY6 F1MMY6_BOVIN;sp Q3SYU7 TNPO1_BOVIN;tr F1MBJ7 F1MBJ7_BOVIN;tr Q2KI57 Q2KI57_BOVIN	22.630	NaN	EV fraction only	1.80E+05
Vesicle-associated membrane protein-associated protein B	sp A2VDZ9 VAPB_BOVIN;sp Q0VCY1 VAPA_BOVIN	22.634	NaN	EV fraction only	1.20E+06
Protein S100-A14	sp Q3MHP3 S10AE_BOVIN	22.636	NaN	EV fraction only	8.20E+05
THUMP domain-containing protein 1	sp Q24K03 THUM1_BOVIN	22.677	NaN	EV fraction only	3.00E+05
Malate dehydrogenase, mitochondrial	sp Q32LG3 MDHM_BOVIN;tr G1K1H1 G1K1H1_BOVIN	22.681	NaN	EV fraction only	5.10E+06
Guanine nucleotide-binding protein subunit gamma	tr G3MYD3 G3MYD3_BOVIN;sp P63217 GBG5_BOVIN	22.684	NaN	EV fraction only	1.70E+06
Uncharacterized protein	tr F1MLD0 F1MLD0_BOVIN	22.692	NaN	EV fraction only	1.60E+05
TAR DNA binding protein	tr Q2KJ45 Q2KJ45_BOVIN;tr G3MX91 G3MX91_BOVIN	22.701	NaN	EV fraction only	5.20E+05
Uncharacterized protein (Fragment)	tr F1N2J6 F1N2J6_BOVIN	22.739	NaN	EV fraction only	2.50E+05
ADP-ribosylation factor-like protein 6	sp Q0IIM2 ARL6_BOVIN	22.744	NaN	EV fraction only	5.90E+05
Tubulin-specific chaperone C	sp Q3SZE9 TBCC_BOVIN	22.751	NaN	EV fraction only	4.20E+05

Isoform 2 of 26S proteasome non-ATPase regulatory subunit 11	sp Q2KI42- 2 PSD11_BOVIN;sp Q2KI42 PS D11_BOVIN	22.767	NaN	EV fraction only	3.00E+05
LANCL2 protein	tr A6QPG6 A6QPG6_BOVIN	22.782	NaN	EV fraction only	3.00E+06
GTP-binding protein Rheb	tr F2Z4I1 F2Z4I1_BOVIN;sp Q56 JV3 RHEB_BOVIN	22.784	NaN	EV fraction only	8.00E+05
Uncharacterized protein (Fragment)	tr E1BG62 E1BG62_BOVIN	22.785	NaN	EV fraction only	2.30E+05
Diamine acetyltransferase 2	sp Q7PCJ8 SAT2_BOVIN	22.801	NaN	EV fraction only	7.30E+05
ATP-dependent (S)- NAD(P)H-hydrate dehydratase	sp E1BNQ4 INNRD_BOVIN	22.802	NaN	EV fraction only	3.70E+05
Uncharacterized protein	tr F1N579 F1N579_BOVIN;tr F1 N4F8 F1N4F8_BOVIN;tr F1MYK 3 F1MYK3_BOVIN	22.833	NaN	EV fraction only	2.10E+06
Serine/threonine-protein phosphatase (Fragment)	tr F1N6B7 F1N6B7_BOVIN	22.835	NaN	EV fraction only	4.20E+05
Metalloproteinase inhibitor 3	tr A5PKB4 A5PKB4_BOVIN;sp P 79121 TIMP3_BOVIN	22.839	NaN	EV fraction only	4.60E+06
Lysosome-associated membrane glycoprotein 1	sp Q05204 LAMP1_BOVIN	22.863	NaN	EV fraction only	3.80E+05
Syntaxin-binding protein 1	sp P61763 STXB1_BOVIN	22.908	NaN	EV fraction only	2.50E+05
Guanidinoacetate N- methyltransferase	sp Q2TBQ3 GAMT_BOVIN	22.914	NaN	EV fraction only	7.90E+05
TNPO3 protein	tr A5D7C4 A5D7C4_BOVIN	22.920	NaN	EV fraction only	7.70E+05

26S proteasome non-ATPase regulatory subunit 12	sp Q2KJ25 PSD12_BOVIN	22.921	NaN	EV fraction only	1.60E+06
Ubiquitin-conjugating enzyme E2 H	sp Q32LN1 UBE2H_BOVIN	22.922	NaN	EV fraction only	1.10E+06
Uncharacterized protein	tr F1MF68 F1MF68_BOVIN;tr G3MZH4 G3MZH4_BOVIN	22.933	NaN	EV fraction only	2.90E+06
Uncharacterized protein	tr F1MBY5 F1MBY5_BOVIN	22.942	NaN	EV fraction only	2.10E+05
Uncharacterized protein	tr F1MXF3 F1MXF3_BOVIN;tr F1MGT1 F1MGT1_BOVIN	22.962	NaN	EV fraction only	1.90E+05
Uncharacterized protein	tr A1A4I8 A1A4I8_BOVIN	22.977	NaN	EV fraction only	1.50E+05
Uncharacterized protein	tr F1MSJ9 F1MSJ9_BOVIN	22.977	NaN	EV fraction only	6.40E+06
Cysteine and glycine-rich protein 1	sp Q3MHY1 CSRP1_BOVIN	22.979	NaN	EV fraction only	6.90E+05
60S ribosomal protein L7a	sp Q2TBQ5 RL7A_BOVIN	22.985	NaN	EV fraction only	8.30E+05
CUL2 protein	tr Q08DE9 Q08DE9_BOVIN	22.995	NaN	EV fraction only	2.00E+05
CTP synthase 2	sp Q1RMS2 PYRG2_BOVIN;tr A0JNE9 A0JNE9_BOVIN	23.000	NaN	EV fraction only	2.60E+05
PGRMC2 protein	tr A5Pjq6 A5Pjq6_BOVIN	23.007	NaN	EV fraction only	1.10E+06
O-acetyl-ADP-ribose deacetylase 1	sp Q1LZ74 OARD1_BOVIN	23.010	NaN	EV fraction only	8.40E+05

Fibronectin type III and SPRY domain-containing protein 1	sp Q05B84 FSD1_BOVIN	23.011	NaN	EV fraction only	2.20E+06
Uncharacterized protein	tr F1MYV9 F1MYV9_BOVIN	23.018	NaN	EV fraction only	3.50E+05
Bisphosphoglycerate mutase	tr F1MX69 F1MX69_BOVIN;sp Q3T014 PMGE_BOVIN	23.019	NaN	EV fraction only	2.80E+06
Uncharacterized protein	tr F1MIC9 F1MIC9_BOVIN	23.021	NaN	EV fraction only	5.20E+06
Sulfotransferase 1A1	tr E1B9K8 E1B9K8_BOVIN;sp P50227 ST1A1_BOVIN	23.026	NaN	EV fraction only	4.40E+06
Ras-related protein Rab-4A	sp Q2TBH7 RAB4A_BOVIN	23.052	NaN	EV fraction only	6.20E+05
Inositol-tetrakisphosphate 1-kinase	sp P0C0T1 ITPK1_BOVIN	23.066	NaN	EV fraction only	5.50E+05
Uncharacterized protein	tr G3N126 G3N126_BOVIN;tr G3N3E4 G3N3E4_BOVIN;tr E1BB91 E1BB91_BOVIN	23.068	NaN	EV fraction only	3.30E+05
Uncharacterized protein	tr G3X787 G3X787_BOVIN	23.076	NaN	EV fraction only	9.60E+06
Uncharacterized protein	tr F1MYP4 F1MYP4_BOVIN;sp Q0VCCQ1 CTBP2_BOVIN;tr F1N053 F1N053_BOVIN;sp Q0VCCQ1-2 CTBP2_BOVIN	23.079	NaN	EV fraction only	4.90E+05
Ras-related protein Rap-1A	sp P62833 RAP1A_BOVIN;sp P61223 RAP1B_BOVIN	23.080	NaN	EV fraction only	5.50E+06
Uncharacterized protein	tr F1MWE0 F1MWE0_BOVIN	23.082	NaN	EV fraction only	9.50E+05

26S proteasome non-ATPase regulatory subunit 13 (Fragment)	tr G8JKV6 G8JKV6_BOVIN;sp Q5E964 PSD13_BOVIN	23.091	NaN	EV fraction only	1.40E+06
N-alpha-acetyltransferase 50	sp Q0IIJ0 NAA50_BOVIN	23.092	NaN	EV fraction only	4.30E+06
V-type proton ATPase subunit D	tr F1N270 F1N270_BOVIN;sp P39942 VATD_BOVIN	23.094	NaN	EV fraction only	6.90E+05
Uncharacterized protein (Fragment)	tr G3MXE4 G3MXE4_BOVIN;tr E1BG60 E1BG60_BOVIN	23.097	NaN	EV fraction only	1.60E+05
Eukaryotic translation initiation factor 4E	sp Q9N0T5 IF4E_BOVIN;tr F1N030 F1N030_BOVIN	23.100	NaN	EV fraction only	1.10E+07
Adenylyl cyclase-associated protein	tr F1N715 F1N715_BOVIN	23.114	NaN	EV fraction only	3.00E+05
Inosine triphosphate pyrophosphatase	sp Q2KIC5 ITPA_BOVIN	23.152	NaN	EV fraction only	9.30E+05
Barrier-to-autointegration factor	sp P61283 BAF_BOVIN	23.183	NaN	EV fraction only	1.60E+06
26S protease regulatory subunit 8	sp P62194 PRS8_BOVIN	23.196	NaN	EV fraction only	4.00E+05
UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase-like 4	tr Q0IIK7 Q0IIK7_BOVIN	23.202	NaN	EV fraction only	1.50E+06
Putative tyrosine-protein phosphatase auxilin	tr F1MPP5 F1MPP5_BOVIN;sp Q27974 AUX1_BOVIN;tr F1MIB2 F1MIB2_BOVIN	23.203	NaN	EV fraction only	2.30E+05
Uncharacterized protein	tr G3N0W8 G3N0W8_BOVIN	23.208	NaN	EV fraction only	5.40E+06

Interferon-induced protein with tetratricopeptide repeats 5	tr Q17QZ9 Q17QZ9_BOVIN	23.209	NaN	EV fraction only	4.20E+05
Protein transport protein Sec23A	tr F1MVW5 F1MVW5_BOVIN;sp A2VDL8 SC23A_BOVIN	23.219	NaN	EV fraction only	4.70E+06
TP53RK protein	tr A5PK80 A5PK80_BOVIN	23.229	NaN	EV fraction only	7.60E+05
Uncharacterized protein	tr F1MEW4 F1MEW4_BOVIN	23.245	NaN	EV fraction only	1.90E+06
Porphobilinogen deaminase	sp Q2KIN5 HEM3_BOVIN	23.254	NaN	EV fraction only	1.90E+07
Plastin-3	sp A7E3Q8 PLST_BOVIN;tr F1MSB7 F1MSB7_BOVIN;sp A6H742 PLSI_BOVIN	23.270	NaN	EV fraction only	1.40E+06
AP-2 complex subunit mu	sp Q3ZC13 AP2M1_BOVIN	23.281	NaN	EV fraction only	1.60E+06
GNB5 protein	tr A5PJS1 A5PJS1_BOVIN	23.300	NaN	EV fraction only	2.50E+06
Uncharacterized protein (Fragment)	tr F6RSR1 F6RSR1_BOVIN	23.303	NaN	EV fraction only	8.50E+06
Ubiquitin carboxyl-terminal hydrolase (Fragment)	tr F1N1Z2 F1N1Z2_BOVIN	23.317	NaN	EV fraction only	1.50E+05
Protein FAM160B1	tr G3X6C8 G3X6C8_BOVIN;sp A0JNG7 F16B1_BOVIN	23.326	NaN	EV fraction only	2.80E+05
Uncharacterized protein	tr G3N1T2 G3N1T2_BOVIN	23.336	NaN	EV fraction only	8.10E+05
Vacuolar protein-sorting-associated protein 36	tr F1MT69 F1MT69_BOVIN;sp A5PK00 VPS36_BOVIN	23.339	NaN	EV fraction only	4.40E+05

Uncharacterized protein (Fragment)	tr F1MYN0 F1MYN0_BOVIN	23.346	NaN	EV fraction only	1.70E+07
Proteasome inhibitor PI31 subunit	sp Q3SX30 PSMF1_BOVIN	23.357	NaN	EV fraction only	1.10E+06
Uncharacterized protein	tr F1N1L4 F1N1L4_BOVIN	23.364	NaN	EV fraction only	7.50E+05
Uncharacterized protein (Fragment)	tr E1BIU7 E1BIU7_BOVIN	23.370	NaN	EV fraction only	1.40E+05
Ceramide synthase 4	sp Q5E9R6 CERS4_BOVIN	23.371	NaN	EV fraction only	7.80E+06
cAMP-dependent protein kinase catalytic subunit alpha	sp P00517 KAPCA_BOVIN	23.377	NaN	EV fraction only	6.10E+05
Uncharacterized protein	tr F2Z4F0 F2Z4F0_BOVIN;sp A4IFE3 ACTY_BOVIN;tr G3N132 G3N132_BOVIN	23.385	NaN	EV fraction only	5.50E+05
Isoform 1 of Aquaporin-4	sp O77750-2 AQP4_BOVIN;sp O77750 AQP4_BOVIN	23.400	NaN	EV fraction only	1.80E+06
Uncharacterized protein (Fragment)	tr F1MNP9 F1MNP9_BOVIN;tr G3N1N5 G3N1N5_BOVIN	23.401	NaN	EV fraction only	1.20E+06
Uncharacterized protein	tr F1MNK0 F1MNK0_BOVIN	23.411	NaN	EV fraction only	1.00E+06
Uncharacterized protein	tr F2Z4H6 F2Z4H6_BOVIN	23.412	NaN	EV fraction only	1.10E+07
Isoform Smooth muscle of Myosin light polypeptide 6	sp P60661-2 MYL6_BOVIN;sp P60661 MYL6_BOVIN	23.419	NaN	EV fraction only	6.70E+06

Olfactomedin-like protein 2B	sp A6QLD2 OLM2B_BOVIN	23.440	NaN	EV fraction only	2.70E+06
Adenosylhomocysteinase	tr A6QLP2 A6QLP2_BOVIN	23.441	NaN	EV fraction only	3.90E+05
Sorting nexin-5	sp Q3ZBM5 SNX5_BOVIN	23.441	NaN	EV fraction only	4.70E+05
Uncharacterized protein (Fragment)	tr F1MIC3 F1MIC3_BOVIN	23.450	NaN	EV fraction only	1.40E+06
Uncharacterized protein	tr E1B8Q8 E1B8Q8_BOVIN	23.471	NaN	EV fraction only	2.60E+05
ERI1 exoribonuclease 3	tr F1ML00 F1ML00_BOVIN;sp A6QLH5 ERI3_BOVIN	23.482	NaN	EV fraction only	1.30E+06
Uncharacterized protein	tr F1N1X8 F1N1X8_BOVIN	23.497	NaN	EV fraction only	8.50E+05
Eukaryotic translation initiation factor 3 subunit M	sp Q3T148 EIF3M_BOVIN;tr F1N5F7 F1N5F7_BOVIN	23.500	NaN	EV fraction only	3.30E+06
ELAV (Embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)	tr A2VDK5 A2VDK5_BOVIN;tr F1N4V6 F1N4V6_BOVIN;tr G3N063 G3N063_BOVIN	23.502	NaN	EV fraction only	6.60E+05
ATCAY protein	tr A2VE75 A2VE75_BOVIN;tr F1N6R4 F1N6R4_BOVIN	23.510	NaN	EV fraction only	9.20E+05
ADP-ribosylation factor-like protein 2	sp Q2TA37 ARL2_BOVIN	23.512	NaN	EV fraction only	1.30E+06
Cerebellin-1	sp P86437 CBLN1_BOVIN	23.523	NaN	EV fraction only	1.50E+07
Uncharacterized protein	tr F1MWB9 F1MWB9_BOVIN	23.548	NaN	EV fraction only	2.00E+07

Uncharacterized protein	tr F1MHR4 F1MHR4_BOVIN;tr F1MHT2 F1MHT2_BOVIN	23.555	NaN	EV fraction only	6.80E+05
Ankyrin repeat and MYND domain-containing protein 2	sp Q0VCS9 ANKY2_BOVIN	23.555	NaN	EV fraction only	5.40E+05
Serine carboxypeptidase 1	tr Q2NKZ9 Q2NKZ9_BOVIN	23.563	NaN	EV fraction only	1.60E+06
Proteasome (Prosome, macropain) 26S subunit, ATPase, 1	tr A4FUZ3 A4FUZ3_BOVIN	23.564	NaN	EV fraction only	2.00E+06
Uncharacterized protein	tr F1N3I3 F1N3I3_BOVIN;tr A3KMX3 A3KMX3_BOVIN	23.571	NaN	EV fraction only	2.30E+05
Uncharacterized protein (Fragment)	tr F1MHQ3 F1MHQ3_BOVIN;tr A7YY64 A7YY64_BOVIN	23.574	NaN	EV fraction only	1.70E+06
PI-PLC X domain-containing protein 3	tr F1N4B7 F1N4B7_BOVIN;sp A6QNU9 PLCX3_BOVIN	23.579	NaN	EV fraction only	1.00E+06
Active breakpoint cluster region-related protein	sp A6QNS3 ABR_BOVIN;sp A6QNS3-2 ABR_BOVIN;tr F1MBT5 F1MBT5_BOVIN	23.582	NaN	EV fraction only	2.90E+05
1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase beta-1	sp P10894 PLCB1_BOVIN	23.600	NaN	EV fraction only	6.60E+05
Uncharacterized protein (Fragment)	tr F1MX04 F1MX04_BOVIN	23.613	NaN	EV fraction only	2.00E+05
Fructose-1,6-bisphosphatase 1	sp Q3SZB7 F16P1_BOVIN	23.615	NaN	EV fraction only	7.10E+05
Ethanolamine-phosphate cytidyltransferase	sp Q5EA75 PCY2_BOVIN	23.626	NaN	EV fraction only	5.40E+05

Signal transducer and activator of transcription	tr E1BPP7 E1BPP7_BOVIN	23.631	NaN	EV fraction only	2.10E+05
Histone H1.2	sp P02253 H12_BOVIN;sp G3N131 H11_BOVIN;tr G3MWW5 G3MWW5_BOVIN;sp A7MAZ5 H13_BOVIN	23.632	NaN	EV fraction only	1.40E+06
Uncharacterized protein	tr E1BJP5 E1BJP5_BOVIN	23.636	NaN	EV fraction only	1.00E+07
Uncharacterized protein	tr E1BIB4 E1BIB4_BOVIN	23.661	NaN	EV fraction only	6.60E+05
MAPK10 protein	tr A4FV00 A4FV00_BOVIN;tr Q2KI72 Q2KI72_BOVIN;tr F1MM73 F1MM73_BOVIN	23.663	NaN	EV fraction only	1.50E+06
Developmentally-regulated GTP-binding protein 2	sp Q58D56 DRG2_BOVIN	23.668	NaN	EV fraction only	6.30E+05
Calponin-3	sp Q32L92 CNN3_BOVIN;sp Q3SYU6 CNN2_BOVIN	23.678	NaN	EV fraction only	8.90E+05
UNC45A protein	tr A5PKJ5 A5PKJ5_BOVIN	23.678	NaN	EV fraction only	2.50E+05
Eukaryotic translation initiation factor 2 subunit 3	sp Q2KHU8 IF2G_BOVIN;tr G3N0A9 G3N0A9_BOVIN;tr G3MWU5 G3MWU5_BOVIN	23.680	NaN	EV fraction only	2.40E+06
Septin-5 (Fragment)	tr F1MEZ1 F1MEZ1_BOVIN;sp Q0VC68 SEPT5_BOVIN	23.688	NaN	EV fraction only	6.40E+05
Retinoid isomerohydrolase	sp Q28175 RPE65_BOVIN	23.692	NaN	EV fraction only	6.20E+05
COP9 signalosome complex subunit 7b	sp Q2KI56 CSN7B_BOVIN	23.696	NaN	EV fraction only	6.50E+06

COPS8 protein	tr A4FV74 A4FV74_BOVIN	23.706	NaN	EV fraction only	1.70E+06
Uncharacterized protein	tr E1BNY9 E1BNY9_BOVIN	23.717	NaN	EV fraction only	1.00E+06
Uncharacterized protein	tr F1MU99 F1MU99_BOVIN	23.721	NaN	EV fraction only	3.50E+07
Eukaryotic translation initiation factor 2 subunit 1	sp P68102 IF2A_BOVIN	23.731	NaN	EV fraction only	1.00E+07
Uncharacterized protein (Fragment)	tr F1MVZ2 F1MVZ2_BOVIN;tr F1MXK4 F1MXK4_BOVIN	23.734	NaN	EV fraction only	3.60E+05
Voltage-dependent anion-selective channel protein 2	sp P68002 VDAC2_BOVIN	23.736	NaN	EV fraction only	8.70E+05
Eukaryotic translation initiation factor 1B	tr Q32LJ9 Q32LJ9_BOVIN;sp Q5E938 EIF1_BOVIN	23.740	NaN	EV fraction only	2.80E+06
Uncharacterized protein	tr E1BA29 E1BA29_BOVIN;sp P38409 GNA11_BOVIN;sp P38408 GNA14_BOVIN;tr G5E6P3 G5E6P3_BOVIN;tr G3N0K2 G3N0K2_BOVIN;tr E1BIL1 E1BIL1_BOVIN	23.747	NaN	EV fraction only	7.40E+05
Ras suppressor protein 1	sp Q5E9C0 RSU1_BOVIN	23.753	NaN	EV fraction only	1.10E+06
Ubiquitin-fold modifier-conjugating enzyme 1	sp Q5E953 UFC1_BOVIN	23.761	NaN	EV fraction only	9.50E+06
Ubiquitin-like domain-containing CTD phosphatase 1	sp Q2KJD7 UBCP1_BOVIN	23.772	NaN	EV fraction only	1.90E+06

Protein O-linked mannose beta1,2-N- acetylglucosaminyltransferase	tr Q3T015 Q3T015_BOVIN;sp Q5EAB6 PMGT1_BOVIN	23.772	NaN	EV fraction only	1.00E+06
Uncharacterized protein	tr F1MWU9 F1MWU9_BOVIN	23.784	NaN	EV fraction only	4.00E+05
Uncharacterized protein	tr F1MY44 F1MY44_BOVIN;tr F1MY12 F1MY12_BOVIN	23.785	NaN	EV fraction only	3.10E+05
Uncharacterized protein	tr G3MZE2 G3MZE2_BOVIN	23.790	NaN	EV fraction only	5.60E+05
Isochorismatase domain- containing protein 1	sp A6QLY4 ISOC1_BOVIN	23.796	NaN	EV fraction only	6.30E+06
Uncharacterized protein	tr F1MSB5 F1MSB5_BOVIN	23.807	NaN	EV fraction only	1.00E+06
Uncharacterized protein (Fragment)	tr G5E5T2 G5E5T2_BOVIN;tr A4IFF4 A4IFF4_BOVIN;tr F1MZW8 F1MZW8_BOVIN	23.808	NaN	EV fraction only	5.40E+05
LRRC24 protein	tr A4IFI5 A4IFI5_BOVIN	23.812	NaN	EV fraction only	6.40E+05
60S ribosomal protein L14	tr G8JKV5 G8JKV5_BOVIN;sp Q3T0U2 RL14_BOVIN	23.815	NaN	EV fraction only	3.00E+06
Leukocyte elastase inhibitor	sp Q1JPB0 ILEU_BOVIN;tr G1K1L8 G1K1L8_BOVIN;sp Q5BIR5 SPB8_BOVIN;tr A6QPW6 A6QPW6_BOVIN	23.821	NaN	EV fraction only	2.60E+06
Uncharacterized protein (Fragment)	tr F1MM21 F1MM21_BOVIN;sp O46470 RGST7_BOVIN	23.822	NaN	EV fraction only	6.40E+05
Uncharacterized protein	tr F6QYV9 F6QYV9_BOVIN	23.823	NaN	EV fraction only	4.90E+05

Uncharacterized protein (Fragment)	tr F1MCI9 F1MCI9_BOVIN	23.835	NaN	EV fraction only	2.80E+05
Uncharacterized protein	tr E1BA27 E1BA27_BOVIN	23.836	NaN	EV fraction only	1.30E+06
Importin subunit alpha-7 (Fragment)	tr G5E536 G5E536_BOVIN;sp Q0V7M0 IMA7_BOVIN;tr F1N1K5 F1N1K5_BOVIN;sp A2VE08 IMA5_BOVIN	23.866	NaN	EV fraction only	6.60E+05
		23.904	NaN	EV fraction only	6.40E+05
Uncharacterized protein	tr F1N1G7 F1N1G7_BOVIN	23.908	NaN	EV fraction only	4.80E+05
Protein kinase C alpha type 2	sp P04409 KPCA_BOVIN;tr F1MJX9 F1MJX9_BOVIN;sp P05126 KPCB_BOVIN;sp P05126-2 KPCB_BOVIN	23.910	NaN	EV fraction only	1.40E+06
Ras-related protein Rab-3C	tr E1BF18 E1BF18_BOVIN;sp P10949 RAB3C_BOVIN	23.914	NaN	EV fraction only	4.80E+05
ACSS2 protein	tr A7YWF1 A7YWF1_BOVIN	23.915	NaN	EV fraction only	2.30E+06
Rhodopsin	sp P02699 OPSD_BOVIN	23.924	NaN	EV fraction only	5.10E+05
Uncharacterized protein	tr F1N4V5 F1N4V5_BOVIN	23.926	NaN	EV fraction only	2.10E+06
UBX domain-containing protein 6	tr F1MES6 F1MES6_BOVIN;sp Q2KIJ6 UBXN6_BOVIN	23.932	NaN	EV fraction only	8.40E+05
TWF2 protein	tr A2VDX0 A2VDX0_BOVIN	23.936	NaN	EV fraction only	1.30E+06
Uncharacterized protein	tr F1MK10 F1MK10_BOVIN				

ACP1 protein	tr A5PK96 A5PK96_BOVIN;sp P11064 PPAC_BOVIN	23.940	NaN	EV fraction only	2.40E+07
Tubulin-folding cofactor B	sp Q5E951 TBCB_BOVIN	23.958	NaN	EV fraction only	7.20E+06
Serine/arginine-rich splicing factor 2	sp Q3MHR5 SRSF2_BOVIN	23.961	NaN	EV fraction only	1.80E+06
Tubulin alpha-8 chain	sp Q2HJB8 TBA8_BOVIN	23.962	NaN	EV fraction only	7.80E+05
Calmegin	sp Q3SYT6 CLGN_BOVIN	23.967	NaN	EV fraction only	4.70E+05
CD9 antigen (Fragment)	tr G8JKX6 G8JKX6_BOVIN;sp P30932 CD9_BOVIN	23.980	NaN	EV fraction only	2.80E+06
Uncharacterized protein	tr F1MLY0 F1MLY0_BOVIN	23.986	NaN	EV fraction only	1.80E+05
C8G protein	tr A8YXZ2 A8YXZ2_BOVIN	24.000	NaN	EV fraction only	2.20E+07
RPRD1B protein	tr A6QLW3 A6QLW3_BOVIN;sp Q0P5J9-Q0P5J9-2 RPR1A_BOVIN;sp Q0P5J9 RP R1A_BOVIN	24.000	NaN	EV fraction only	1.20E+06
Uncharacterized protein	tr F1MS62 F1MS62_BOVIN;tr G3MZI2 G3MZI2_BOVIN	24.004	NaN	EV fraction only	1.10E+06
Uncharacterized protein	tr F1N2Q9 F1N2Q9_BOVIN	24.007	NaN	EV fraction only	2.50E+06
Protein Hikeshi	sp Q56JY0 HIKES_BOVIN	24.016	NaN	EV fraction only	3.40E+06
Uncharacterized protein	tr E1BDS4 E1BDS4_BOVIN	24.024	NaN	EV fraction only	1.60E+07

Midkine	tr Q9N0E6 Q9N0E6_BOVIN	24.039	NaN	EV fraction only	2.50E+06
Protein prune homolog	sp Q5E9Y6 PRUNE_BOVIN	24.040	NaN	EV fraction only	7.20E+05
Uncharacterized protein	tr F1MS94 F1MS94_BOVIN	24.042	NaN	EV fraction only	1.40E+06
TBC1 domain family member 24	tr F1N534 F1N534_BOVIN;sp Q29RJ2 TBC24_BOVIN	24.046	NaN	EV fraction only	6.40E+05
AP2-associated protein kinase 1 (Fragment)	tr H2XJE8 H2XJE8_BOVIN;sp F1MH24 AAK1_BOVIN	24.075	NaN	EV fraction only	4.80E+05
Pre-B-cell leukemia transcription factor-interacting protein 1	sp A6QLY7 PBIP1_BOVIN	24.090	NaN	EV fraction only	6.40E+05
Uncharacterized protein	tr F1MYJ4 F1MYJ4_BOVIN;tr G3X7B5 G3X7B5_BOVIN;sp Q01314 AKT1_BOVIN	24.093	NaN	EV fraction only	5.60E+06
Isoform 2 of Catechol O-methyltransferase	sp A7MBI7-2 COMT_BOVIN;sp A7MBI7 COMT_BOVIN	24.106	NaN	EV fraction only	1.40E+06
Heterogeneous nuclear ribonucleoprotein H2	tr A5D9B4 A5D9B4_BOVIN;sp Q3SZF3 HNRH2_BOVIN;tr E1BF20 E1BF20_BOVIN	24.107	NaN	EV fraction only	1.30E+07
Vacuolar protein sorting-associated protein VTA1 homolog	tr F1N318 F1N318_BOVIN;sp Q32L63 VTA1_BOVIN	24.115	NaN	EV fraction only	1.50E+06
Mannose-1-phosphate guanylttransferase beta	tr F1N7H5 F1N7H5_BOVIN;sp Q2YDJ9 GMPPB_BOVIN	24.124	NaN	EV fraction only	1.10E+06

Signal transducer and activator of transcription 5B	sp Q9TUM3 STA5B_BOVIN;sp Q95115 STA5A_BOVIN;tr F1MER9_BOVIN	24.130	NaN	EV fraction only	4.70E+05
Excitatory amino acid transporter 1	sp P46411 EAA1_BOVIN	24.145	NaN	EV fraction only	1.20E+06
GNAI2 protein	tr A7MBH9 A7MBH9_BOVIN;tr Q3ZCA7 Q3ZCA7_BOVIN;sp P63097 GNAI1_BOVIN	24.153	NaN	EV fraction only	1.10E+06
Haloacid dehalogenase-like hydrolase domain containing 1A	tr Q2KJ86 Q2KJ86_BOVIN	24.160	NaN	EV fraction only	1.60E+06
Protein FAM49A	sp Q17QT7 FA49A_BOVIN	24.161	NaN	EV fraction only	1.00E+06
Uncharacterized protein	tr F1MMA0 F1MMA0_BOVIN;sp Q08E27 STRBP_BOVIN	24.172	NaN	EV fraction only	5.30E+05
Uncharacterized protein	tr E1BM42 E1BM42_BOVIN	24.173	NaN	EV fraction only	3.30E+05
MGAT1 protein	tr Q5E9I4 Q5E9I4_BOVIN	24.181	NaN	EV fraction only	9.50E+05
UPF0468 protein C16orf80 homolog	sp Q6B857 CP080_BOVIN	24.191	NaN	EV fraction only	1.60E+06
Uncharacterized protein	tr E1BNG8 E1BNG8_BOVIN;tr Q2KJ23 Q2KJ23_BOVIN	24.245	NaN	EV fraction only	1.90E+06
Uncharacterized protein (Fragment)	tr F1MHJ5 F1MHJ5_BOVIN;tr F1MR64 F1MR64_BOVIN	24.265	NaN	EV fraction only	9.20E+05
ADP-ribosylation factor 1	sp P84080 ARF1_BOVIN	24.266	NaN	EV fraction only	2.00E+06
Uncharacterized protein (Fragment)	tr F1MFT4 F1MFT4_BOVIN	24.269	NaN	EV fraction only	5.90E+05

Uncharacterized protein	tr F1N2D5 F1N2D5_BOVIN	24.272	NaN	EV fraction only	7.50E+06
Uncharacterized protein	tr E1B9H5 E1B9H5_BOVIN	24.287	NaN	EV fraction only	1.40E+06
PLAA protein	tr A7Z055 A7Z055_BOVIN	24.288	NaN	EV fraction only	4.50E+05
Integrin beta-2	sp P32592 ITB2_BOVIN	24.290	NaN	EV fraction only	3.80E+06
Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3	sp A7YY55 HACD3_BOVIN	24.297	NaN	EV fraction only	1.10E+06
Uncharacterized protein	tr F1MJ80 F1MJ80_BOVIN	24.311	NaN	EV fraction only	7.20E+05
MOB1, Mps One Binder kinase activator-like 1B (Yeast)	tr Q0VCJ5 Q0VCJ5_BOVIN;tr E1BMG1 E1BMG1_BOVIN	24.315	NaN	EV fraction only	2.10E+06
Protein FAM49B	sp Q2KJ13 FA49B_BOVIN	24.332	NaN	EV fraction only	1.10E+06
Isoform 2 of Serine/threonine-protein phosphatase 2B catalytic subunit alpha isoform	sp P48452-2 PP2BA_BOVIN;sp P48452 PP2BA_BOVIN;tr G3MYK1 G3MYK1_BOVIN	24.348	NaN	EV fraction only	3.90E+06
Uncharacterized protein	tr F1N7U2 F1N7U2_BOVIN	24.352	NaN	EV fraction only	7.40E+05
FTO protein	tr A5D798 A5D798_BOVIN	24.363	NaN	EV fraction only	7.20E+05
Annexin A2	sp P04272 ANXA2_BOVIN	24.372	NaN	EV fraction only	9.40E+05

Uncharacterized protein	tr F1MX43 F1MX43_BOVIN	24.381	NaN	EV fraction only	1.80E+06
Uncharacterized protein	tr F2Z4E7 F2Z4E7_BOVIN	24.382	NaN	EV fraction only	1.20E+06
Protein NDRG3	sp A7MB28 NDRG3_BOVIN	24.385	NaN	EV fraction only	1.50E+06
Ribosome maturation protein SBDS	sp Q3SWZ6 SBDS_BOVIN	24.390	NaN	EV fraction only	1.30E+06
Uncharacterized protein	tr E1BMX0 E1BMX0_BOVIN;tr G3MXH2 G3MXH2_BOVIN;tr A5D7R9 A5D7R9_BOVIN;tr G3N1U2 G3N1U2_BOVIN	24.400	NaN	EV fraction only	2.50E+06
Uncharacterized protein (Fragment)	tr F1MSM4 F1MSM4_BOVIN	24.403	NaN	EV fraction only	1.10E+06
Transcription elongation factor B polypeptide 1	sp Q2KI4 ELOC_BOVIN	24.410	NaN	EV fraction only	9.50E+06
Uncharacterized protein	tr F6QE33 F6QE33_BOVIN;tr F1MDK0 F1MDK0_BOVIN	24.413	NaN	EV fraction only	1.50E+06
Uncharacterized protein (Fragment)	tr F1MX83 F1MX83_BOVIN	24.437	NaN	EV fraction only	2.90E+07
Uncharacterized protein	tr E1B763 E1B763_BOVIN	24.442	NaN	EV fraction only	1.00E+06
Monocarboxylate transporter 1	sp Q3MHW6 MOT1_BOVIN	24.442	NaN	EV fraction only	1.60E+06
>P07477 SWISS-PROT:P07477 Tax_Id=9606 Gene_Symbol=PRSS1 Trypsin-1 precursor	CON_P07477	24.461	NaN	EV fraction only	9.50E+06

Uncharacterized protein (Fragment)	tr F1N025 F1N025_BOVIN	24.470	NaN	EV fraction only	8.00E+05
Uncharacterized protein (Fragment)	tr F1N6Y7 F1N6Y7_BOVIN	24.476	NaN	EV fraction only	4.60E+05
BLVRA protein	tr A5D7K0 A5D7K0_BOVIN	24.486	NaN	EV fraction only	1.50E+06
RAB10 protein	tr A6QLS9 A6QLS9_BOVIN	24.489	NaN	EV fraction only	7.00E+06
Uncharacterized protein	tr F1N7F4 F1N7F4_BOVIN	24.520	NaN	EV fraction only	1.20E+07
Prolargin	tr F1MX63 F1MX63_BOVIN;sp Q9GKN8 PRELP_BOVIN	24.537	NaN	EV fraction only	7.30E+06
NHP2-like protein 1	sp Q3B8S0 NH2L1_BOVIN	24.547	NaN	EV fraction only	3.10E+06
Tubulin-specific chaperone E	sp Q32KS0 TBCE_BOVIN	24.561	NaN	EV fraction only	2.10E+06
Uncharacterized protein	tr F1MUP9 F1MUP9_BOVIN	24.563	NaN	EV fraction only	3.60E+06
Lipopolysaccharide-binding protein	tr F1MNN7 F1MNN7_BOVIN;sp Q2TBI0 LBP_BOVIN	24.572	NaN	EV fraction only	1.30E+06
Uncharacterized protein	tr F1MX60 F1MX60_BOVIN;tr E1BN47 E1BN47_BOVIN	24.590	NaN	EV fraction only	3.70E+05
CaM kinase-like vesicle-associated	tr Q08DK8 Q08DK8_BOVIN	24.623	NaN	EV fraction only	3.10E+06
Uncharacterized protein	tr F1MYK4 F1MYK4_BOVIN	24.632	NaN	EV fraction only	1.80E+06
Uncharacterized protein	tr E1BBK6 E1BBK6_BOVIN	24.636	NaN	EV fraction only	4.30E+06

T-complex protein 1 subunit alpha	tr G5E531 G5E531_BOVIN;sp Q32L40 TCPA_BOVIN	24.637	NaN	EV fraction only	5.90E+06
Uncharacterized protein	tr E1BDM8 E1BDM8_BOVIN	24.650	NaN	EV fraction only	9.40E+05
Septin-6	sp Q3SZN0 SEPT6_BOVIN;sp A2VE99 SEP11_BOVIN	24.677	NaN	EV fraction only	4.40E+06
ATP synthase subunit alpha	tr F1MLB8 F1MLB8_BOVIN;sp P19483 ATPA_BOVIN	24.685	NaN	EV fraction only	8.70E+05
TIP41, TOR signaling pathway regulator-like (S. cerevisiae)	tr Q29RT7 Q29RT7_BOVIN	24.690	NaN	EV fraction only	6.50E+06
Leucine carboxyl methyltransferase 1	sp Q3T0H0 LCMT1_BOVIN	24.694	NaN	EV fraction only	3.40E+06
Isoform 2 of Reticulon-3	sp Q08D83-2 RTN3_BOVIN;tr G3X7U3 G3X7U3_BOVIN;tr G8JKY8 G8JKY8_BOVIN;sp Q08D83 RTN3_BOVIN	24.695	NaN	EV fraction only	4.50E+06
Ras-related protein Rab-6B	sp A6QR46 RAB6B_BOVIN	24.697	NaN	EV fraction only	2.10E+06
Monocyte differentiation antigen CD14	sp Q95122 CD14_BOVIN;tr A6QNL0 A6QNL0_BOVIN	24.698	NaN	EV fraction only	4.30E+06
Coronin	tr A2VDN8 A2VDN8_BOVIN;tr G3MWI5 G3MWI5_BOVIN	24.698	NaN	EV fraction only	5.00E+06
KH domain containing, RNA binding, signal transduction associated 1	tr Q29RQ2 Q29RQ2_BOVIN	24.706	NaN	EV fraction only	1.70E+07
Plexin domain containing 2	tr A0JN47 A0JN47_BOVIN	24.707	NaN	EV fraction only	8.50E+06

Annexin	tr F6QVC9 F6QVC9_BOVIN;sp P81287 ANXA5_BOVIN	24.710	NaN	EV fraction only	1.20E+06
AP-2 complex subunit beta	sp P63009 AP2B1_BOVIN;sp Q08DS7 AP1B1_BOVIN;tr G3X7G4 G3X7G4_BOVIN	24.715	NaN	EV fraction only	1.40E+06
Uncharacterized protein (Fragment)	tr E1BLV6 E1BLV6_BOVIN	24.716	NaN	EV fraction only	6.10E+05
Basigin	tr Q3ZBX0 Q3ZBX0_BOVIN	24.718	NaN	EV fraction only	1.80E+06
RAB2A, member RAS oncogene family	tr Q148J4 Q148J4_BOVIN	24.722	NaN	EV fraction only	9.30E+06
Asparagine--tRNA ligase, cytoplasmic	tr F1MKH6 F1MKH6_BOVIN;sp Q2KJG3 SYNC_BOVIN;tr G3MXW8 G3MXW8_BOVIN	24.730	NaN	EV fraction only	3.80E+06
11-cis retinol dehydrogenase	sp Q27979 RDH1_BOVIN	24.740	NaN	EV fraction only	1.60E+06
Uncharacterized protein (Fragment)	tr F1MW66 F1MW66_BOVIN	24.742	NaN	EV fraction only	5.20E+06
Ubiquitin-like modifier-activating enzyme 5	sp A7MAZ3 UBA5_BOVIN	24.742	NaN	EV fraction only	1.70E+06
cAMP-dependent protein kinase type II-beta regulatory subunit	tr F6Q9S4 F6Q9S4_BOVIN;sp P31322 KAP3_BOVIN	24.761	NaN	EV fraction only	1.20E+06
Uncharacterized protein	tr F1MZK4 F1MZK4_BOVIN;tr A6H7D7 A6H7D7_BOVIN	24.788	NaN	EV fraction only	1.00E+06
Suppressor of G2 allele of SKP1 homolog	sp Q2KIK0 SUGT1_BOVIN;tr G1K147 G1K147_BOVIN	24.807	NaN	EV fraction only	3.50E+06
Uncharacterized protein	tr E1BPN4 E1BPN4_BOVIN	24.822	NaN	EV fraction only	4.60E+06

Uncharacterized protein	tr F1MZT1 F1MZT1_BOVIN	24.834	NaN	EV fraction only	5.60E+06
Adenylosuccinate lyase	tr F1MHP6 F1MHP6_BOVIN;sp A3KN12 PUR8_BOVIN	24.837	NaN	EV fraction only	1.80E+06
Ganglioside-induced differentiation-associated protein 1-like 1	tr Q1JPF3 Q1JPF3_BOVIN	24.858	NaN	EV fraction only	1.50E+06
Rhopilin-2	tr F1MZ22 F1MZ22_BOVIN;sp A4FUC9 RHPN2_BOVIN	24.878	NaN	EV fraction only	7.70E+05
Ubiquitin-conjugating enzyme E2 variant 1	sp Q3SZ52 UB2V1_BOVIN	24.885	NaN	EV fraction only	4.80E+07
Cell division control protein 42 homolog	sp Q2KJ93 CDC42_BOVIN;tr F1MND1 F1MND1_BOVIN;tr Q1RM I2 Q1RMI2_BOVIN;tr E1BLE9 E1 BLE9_BOVIN;tr F1MK42 F1MK4 2_BOVIN;tr A4FV20 A4FV20_B OVIN	24.895	NaN	EV fraction only	1.10E+07
Uncharacterized protein	tr E1B820 E1B820_BOVIN	24.916	NaN	EV fraction only	7.40E+05
Annexin	tr F1N650 F1N650_BOVIN;sp P4 6193 ANXA1_BOVIN	24.954	NaN	EV fraction only	1.60E+06
Voltage-dependent anion-selective channel protein 1	sp P45879 VDAC1_BOVIN;tr F1 MIN1 F1MIN1_BOVIN	24.961	NaN	EV fraction only	1.90E+06
Actin-related protein 2/3 complex subunit 3	sp Q3T035 ARPC3_BOVIN	24.975	NaN	EV fraction only	1.70E+07
Uncharacterized protein (Fragment)	tr F1MPZ9 F1MPZ9_BOVIN	24.976	NaN	EV fraction only	5.70E+05
Replication protein A1, 70kDa	tr Q0VCV0 Q0VCV0_BOVIN	24.994	NaN	EV fraction only	1.00E+06

Twinfilin-1	sp Q56JV6 TWF1_BOVIN	24.996	NaN	EV fraction only	1.50E+06
Ras-related protein Rab-5A	sp Q0IIG7 RAB5A_BOVIN;tr G3N2V0 G3N2V0_BOVIN	25.008	NaN	EV fraction only	3.10E+06
				EV fraction only	1.60E+06
DEK protein	tr A5PJQ1 A5PJQ1_BOVIN	25.025	NaN	EV fraction only	2.60E+06
				EV fraction only	2.00E+06
Tripartite motif-containing protein 2	sp A4IF63 TRIM2_BOVIN	25.033	NaN	EV fraction only	7.60E+05
				EV fraction only	3.90E+06
ADP-dependent glucokinase	sp A2VE47 ADPGK_BOVIN	25.035	NaN	EV fraction only	9.00E+05
	tr F1MYD0 F1MYD0_BOVIN;tr G3N0Q3 G3N0Q3_BOVIN	25.050	NaN	EV fraction only	6.70E+06
Uncharacterized protein				EV fraction only	4.20E+06
	sp Q2YDM1 ARL1_BOVIN	25.058	NaN	EV fraction only	2.40E+06
ADP-ribosylation factor-like protein 1	tr G5E631 G5E631_BOVIN;tr D3IVZ2 D3IVZ2_BOVIN	25.069	NaN	EV fraction only	1.30E+06
				EV fraction only	1.40E+06
Proteasome (Prosome, macropain) activator subunit 1 (PA28 alpha)	tr Q2KJE7 Q2KJE7_BOVIN;sp Q4U5R3 PSME1_BOVIN	25.072	NaN	EV fraction only	3.30E+06
				EV fraction only	
Uncharacterized protein	tr E1BIP3 E1BIP3_BOVIN	25.072	NaN	EV fraction only	
				EV fraction only	
Synaptobrevin homolog YKT6	sp Q3T000 YKT6_BOVIN	25.077	NaN	EV fraction only	
				EV fraction only	
Uncharacterized protein	tr F1MM57 F1MM57_BOVIN	25.108	NaN	EV fraction only	
				EV fraction only	
Serine/threonine-protein kinase PAK 1	sp Q08E52 PAK1_BOVIN	25.123	NaN	EV fraction only	
				EV fraction only	
ADP-ribosylation factor-like protein 8B	tr F2Z4I5 F2Z4I5_BOVIN;sp Q2KI07 ARL8B_BOVIN	25.134	NaN	EV fraction only	
				EV fraction only	

Uncharacterized protein	tr F1N5K2 F1N5K2_BOVIN	25.194	NaN	EV fraction only	1.10E+06
Cyclin-dependent kinase 5	sp Q02399 CDK5_BOVIN;tr G3N0Y1 G3N0Y1_BOVIN;sp Q5E9Y0 CDK2_BOVIN;sp Q32KY4 CDK4_BOVIN;tr A5PJJ9 A5PJJ9_BOVIN;tr E1BC36 E1BC36_BOVIN;sp Q5EAB2 CDK9_BOVIN;tr A3KMY7 A3KMY7_BOVIN;tr A6QR30 A6QR30_BOVIN;tr F1MN42 F1MN42_BOVIN;sp E1BB50 CDK12_BOVIN;tr G5E518 G5E518_BOVIN;sp E1BB52 CDK13_BOVIN	25.224	NaN	EV fraction only	8.30E+06
	N				
	tr F1MJ53 F1MJ53_BOVIN;tr G3MXD5 G3MXD5_BOVIN;tr F1N061 F1N061_BOVIN	25.248	NaN	EV fraction only	1.30E+06
		25.275	NaN	EV fraction only	3.10E+06
Uncharacterized protein (Fragment) COP9 signalosome complex subunit 4	sp Q3SZA0 CSN4_BOVIN	25.297	NaN	EV fraction only	1.30E+06
KIF1-binding protein	sp Q3SYS9 KBP_BOVIN				
ADP/ATP translocase 2	sp Q8SQH5 ADT2_BOVIN;sp P02722 ADT1_BOVIN;tr G3N3W3 G3N3W3_BOVIN;sp P32007 ADT3_BOVIN;tr F1MDK8 F1MDK8_BOVIN;sp Q2YDD9 ADT4_BOVIN	25.320	NaN	EV fraction only	2.10E+06
	N				
Uncharacterized protein	tr G3N3N1 G3N3N1_BOVIN	25.322	NaN	EV fraction only	5.20E+06

Uncharacterized protein	tr G5E6L2 G5E6L2_BOVIN	25.323	NaN	EV fraction only	9.60E+06
Calcium/calmodulin-dependent protein kinase II delta	tr A5D9F0 A5D9F0_BOVIN;sp Q2HJF7 KCC2D_BOVIN;tr Q08E45 Q08E45_BOVIN	25.326	NaN	EV fraction only	6.20E+06
Uncharacterized protein	tr F6RWK1 F6RWK1_BOVIN	25.327	NaN	EV fraction only	1.50E+06
Uncharacterized protein	tr F1N7B5 F1N7B5_BOVIN	25.336	NaN	EV fraction only	2.00E+06
Uncharacterized protein	tr E1B7J7 E1B7J7_BOVIN	25.346	NaN	EV fraction only	3.60E+06
Neurocalcin-delta	sp P61602 NCALD_BOVIN;sp Q4PL64 HPCA_BOVIN;sp P29105 HPCL1_BOVIN	25.370	NaN	EV fraction only	7.10E+06
Epoxide hydrolase 1, microsomal (Xenobiotic)	tr Q3ZCJ6 Q3ZCJ6_BOVIN	25.388	NaN	EV fraction only	1.50E+06
Uncharacterized protein (Fragment)	tr F1MDV3 F1MDV3_BOVIN	25.400	NaN	EV fraction only	1.30E+06
Uncharacterized protein	tr F1ME38 F1ME38_BOVIN	25.408	NaN	EV fraction only	7.80E+05
Isoform					
Cytoplasmic+peroxisomal of Peroxiredoxin-5, mitochondrial	sp Q9BGI1-2 PRDX5_BOVIN;sp Q9BGI1 PRDX5_BOVIN	25.417	NaN	EV fraction only	1.10E+07
Phosphatidylinositol transfer protein beta isoform	sp Q9TR36 PIPNB_BOVIN	25.423	NaN	EV fraction only	2.50E+06
AP-3 complex subunit delta-1	sp Q865S1 AP3D1_BOVIN	25.448	NaN	EV fraction only	8.30E+05

Ribose-phosphate pyrophosphokinase 1	sp Q2HJ58 PRPS1_BOVIN;tr G3 MY14 G3MY14_BOVIN;tr F6RJ9 1 F6RJ91_BOVIN	25.452	NaN	EV fraction only	1.20E+07
Uncharacterized protein	tr E1BBG4 E1BBG4_BOVIN	25.483	NaN	EV fraction only	7.80E+06
Fibulin 5	tr Q2KJ89 Q2KJ89_BOVIN;sp Q 5EA62 FBLN5_BOVIN	25.499	NaN	EV fraction only	5.00E+06
Prostamide/prostaglandin F synthase	sp Q58CY6 PGFS_BOVIN	25.510	NaN	EV fraction only	4.80E+06
Sulfotransferase family 4A, member 1	tr Q17QV7 Q17QV7_BOVIN	25.526	NaN	EV fraction only	1.60E+07
Ubiquitin-conjugating enzyme E2 K	sp P61085 UBE2K_BOVIN	25.541	NaN	EV fraction only	4.10E+06
MTHFD1 protein	tr A4FUD0 A4FUD0_BOVIN	25.548	NaN	EV fraction only	9.60E+05
Guanylate cyclase soluble subunit alpha-1	sp P19687 GCYA1_BOVIN	25.553	NaN	EV fraction only	1.30E+06
Beta-galactoside alpha-2,6- sialyltransferase 2	sp A5D7T4 SIAT2_BOVIN	25.587	NaN	EV fraction only	4.70E+06
ATP-dependent RNA helicase DDX1	sp Q0IIK5 DDX1_BOVIN	25.590	NaN	EV fraction only	1.80E+06
AMPH protein	tr A5D783 A5D783_BOVIN	25.593	NaN	EV fraction only	3.00E+06
Zinc finger Ran-binding domain-containing protein 2	tr A7YWH2 A7YWH2_BOVIN	25.596	NaN	EV fraction only	2.80E+06
Uncharacterized protein	tr E1BQ15 E1BQ15_BOVIN;tr E1 BGU2 E1BGU2_BOVIN;tr G3N2 Y5 G3N2Y5_BOVIN	25.678	NaN	EV fraction only	1.20E+06

DDX17 protein	tr A7E307 A7E307_BOVIN	25.702	NaN	EV fraction only	2.90E+06
DCLK1 protein	tr A8E644 A8E644_BOVIN;tr G3MYK0 G3MYK0_BOVIN;tr E1BBU3 E1BBU3_BOVIN	25.711	NaN	EV fraction only	5.80E+06
	Interferon-inducible double-stranded RNA-dependent protein kinase activator A			EV fraction only	
Beta-soluble NSF attachment protein	sp Q2HJ92 PRKRA_BOVIN	25.780	NaN	EV fraction only	6.40E+06
	sp P81126 SNAB_BOVIN	25.785	NaN	EV fraction only	5.70E+06
Uncharacterized protein (Fragment)	tr F1MP10 F1MP10_BOVIN	25.809	NaN	EV fraction only	3.30E+06
Sorting nexin-2	tr F1MZN7 F1MZN7_BOVIN;sp Q2TBW7 SNX2_BOVIN;tr F1MYH6 F1MYH6_BOVIN;tr F1N399 F1N399_BOVIN;sp Q05B62 SNX1_BOVIN			EV fraction only	
	Uncharacterized protein (Fragment)	25.824	NaN	EV fraction only	2.20E+06
Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B	tr F1MC86 F1MC86_BOVIN	25.825	NaN	EV fraction only	2.80E+06
	tr F1MG56 F1MG56_BOVIN;sp Q5E9Q7 2ABB_BOVIN;tr F1MY94 F1MY94_BOVIN	25.848	NaN	EV fraction only	4.60E+06
Canx protein	tr A7Z066 A7Z066_BOVIN	25.849	NaN	EV fraction only	1.90E+06
Ataxin-10	tr F1MH20 F1MH20_BOVIN;sp Q2TBW0 ATX10_BOVIN	25.850	NaN	EV fraction only	3.50E+06
	Endoplasmin	25.859	NaN	EV fraction only	3.50E+06

TBCD protein	tr A7MB50 A7MB50_BOVIN;sp Q28205 TBCD_BOVIN	25.865	NaN	EV fraction only	1.40E+06
Sorting nexin-3	sp Q1RMH8 SNX3_BOVIN	25.870	NaN	EV fraction only	8.90E+06
6-phosphofructokinase	tr E1BCW3 E1BCW3_BOVIN	25.876	NaN	EV fraction only	1.90E+06
Histidine triad nucleotide-binding protein 2, mitochondrial	sp Q8SQ21 HINT2_BOVIN	25.896	NaN	EV fraction only	1.60E+07
Uncharacterized protein	tr F6R173 F6R173_BOVIN;tr F1MDA8 F1MDA8_BOVIN	25.896	NaN	EV fraction only	3.50E+06
GTP-binding protein SAR1a	sp Q3T0D7 SAR1A_BOVIN	25.911	NaN	EV fraction only	6.30E+06
MAP2K4 protein	tr A5PJP8 A5PJP8_BOVIN	25.913	NaN	EV fraction only	2.70E+06
MAP2K2 protein	tr Q17QH2 Q17QH2_BOVIN	25.915	NaN	EV fraction only	6.40E+06
ARF5 protein	tr A4IFP7 A4IFP7_BOVIN	25.923	NaN	EV fraction only	1.40E+07
55 kDa erythrocyte membrane protein	sp Q17QN6 EM55_BOVIN	25.958	NaN	EV fraction only	4.10E+06
Guanylate cyclase soluble subunit beta-1	tr G3N145 G3N145_BOVIN;sp P16068 GCYB1_BOVIN	25.974	NaN	EV fraction only	2.40E+06
Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit epsilon isoform	sp A4FV68 2A5E_BOVIN;tr Q08DP7 Q08DP7_BOVIN	25.990	NaN	EV fraction only	6.10E+06

Guanine nucleotide-binding protein G(o) subunit alpha	sp P08239 GNAO_BOVIN;tr G8JKZ5 G8JKZ5_BOVIN;tr F1N461 F1N461_BOVIN;sp P04896-2 GNAS2_BOVIN;sp P04896 GNAS2_BOVIN	26.064	NaN	EV fraction only	1.00E+07
T-complex protein 1 subunit theta	sp Q3ZCI9 TCPQ_BOVIN;tr G3X861 G3X861_BOVIN	26.065	NaN	EV fraction only	8.30E+06
Uncharacterized protein	tr E1BFC3 E1BFC3_BOVIN	26.093	NaN	EV fraction only	5.90E+06
Eukaryotic initiation factor 4A-I	sp Q3SZ54 IF4A1_BOVIN	26.121	NaN	EV fraction only	1.10E+07
V-type proton ATPase subunit C 1	sp P21282 VATC1_BOVIN	26.189	NaN	EV fraction only	4.10E+06
Uncharacterized protein (Fragment)	tr F6RJG0 F6RJG0_BOVIN	26.224	NaN	EV fraction only	4.70E+06
Uncharacterized protein (Fragment)	tr E1BEI0 E1BEI0_BOVIN	26.229	NaN	EV fraction only	7.20E+06
Annexin A6	sp P79134 ANXA6_BOVIN	26.312	NaN	EV fraction only	1.90E+06
tRNA-splicing ligase RtcB homolog	sp Q5E9T9 RTCB_BOVIN	26.380	NaN	EV fraction only	3.60E+06
Uncharacterized protein (Fragment)	tr F1MU05 F1MU05_BOVIN	26.380	NaN	EV fraction only	2.70E+06

	sp Q3ZBX9 H2AJ_BOVIN;tr G8JL00 G8JL00_BOVIN;tr F2Z4J1 F2Z4J1_BOVIN;tr F2Z4G5 F2Z4G5_BOVIN;tr A4IFU5 A4IFU5_BOVIN;sp P0C0S9 H2A1_BOVIN;sp A1A4R1 H2A2C_BOVIN;tr F2Z4I6 F2Z4I6_BOVIN;tr Q17QG8 Q17QG8_BOVIN;sp Q32LA7 H2AV_BOVIN;sp P0C0S4 H2AZ_BOVIN;tr F1MLQ1 F1MLQ1_BOVIN;tr F1MRN2 F1MRN2_BOVIN;tr E1BH22 E1BH22_BOVIN;tr F1MT45 F1MT45_BOVIN	26.413	NaN	EV fraction only	3.10E+07
Histone H2A.J					
Uncharacterized protein	tr E1BGE5 E1BGE5_BOVIN;tr E1B8Q9 E1B8Q9_BOVIN	26.421	NaN	EV fraction only	5.40E+06
COP9 signalosome complex subunit 3			NaN	EV fraction only	5.10E+06
RAP1 GTPase activating protein	sp A6H7B5 CSN3_BOVINtr Q08E64 Q08E64_BOVIN;tr F1N2P1 F1N2P1_BOVIN	26.440	NaN	EV fraction only	5.10E+06
Galactokinase	sp A6H768 GALK1_BOVIN;tr G1K1R6 G1K1R6_BOVIN	26.453	NaN	EV fraction only	5.10E+06
		26.458	NaN	EV fraction only	6.60E+06
NDRG family member 4	tr Q0VCK8 Q0VCK8_BOVIN	26.527	NaN	EV fraction only	9.10E+06
Glutamate--cysteine ligase regulatory subunit	sp Q2T9Y6 GSH0_BOVIN	26.568	NaN	EV fraction only	1.10E+07
AHA1, activator of heat shock 90kDa protein				EV fraction only	
ATPase homolog 1 (Yeast)	tr Q3T0G3 Q3T0G3_BOVIN	26.574	NaN	EV fraction only	1.30E+07

Uncharacterized protein	tr E1BIN5 E1BIN5_BOVIN	26.776	NaN	EV fraction only	3.90E+06
Uncharacterized protein-RPE-retinal G protein-coupled receptor	tr F1N102 F1N102_BOVIN;tr F1MX86 F1MX86_BOVIN	26.837	NaN	EV fraction only	1.00E+07
	sp P47803 RGR_BOVIN	26.918	NaN	EV fraction only	1.80E+07
	Solute carrier family 2, facilitated glucose transporter member 1	26.928	NaN	EV fraction only	1.10E+07
Uncharacterized protein	tr G3N2N1 G3N2N1_BOVIN	26.929	NaN	EV fraction only	9.30E+06

Histone H2B	U F1N433 F1N433_BOVIN;tr E1BGW2 E1BGW2_BOVIN;tr G8JL06 G8JL06_BOVIN;tr E1B8G9 E1B8G9_BOVIN;tr Q32S29 Q32S29_BOVIN;tr Q2KII5 Q2KII5_BOVIN;tr F2Z4F9 F2Z4F9_BOVIN;tr F2Z4E8 F2Z4E8_BOVIN;tr F1MUD2 F1MUD2_BOVIN;sp Q2M2T1 H2B1K_BOVIN;sp P62808 H2B1_BOVIN;tr G3N080 G3N080_BOVIN;tr G3N0F3 G3N0F3_BOVIN;tr F1MIF8 F1MIF8_BOVIN;tr G5E6I9 G5E6I9_BOVIN;tr G3N3L9 G3N3L9_BOVIN;tr G3N1C9 G3N1C9_BOVIN;tr G3N068 G3N068_BOVIN;tr G3MYV4 G3MYV4_BOVIN;tr G3MWH4 G3MWH4_BOVIN;tr F1MUU9 F1MUU9_BOVIN;sp Q32L48 H2B1N_BOVIN;tr G3N053 G3N053_BOVIN;tr E1BK75 E1BK75_BOVIN;tr G3MXP6 G3MXP6_BOVIN;tr F1MV26 F1MV26_BOVIN;tr A6QQ28 A6QQ28_BOVIN;tr G3MZL8 G3MZL8_BOVIN;tr G3MX03 G3MX03_BOVIN;tr	26.997	NaN	EV fraction only	2.90E+07
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>P35908 SWISS- PROT:P35908 Tax_Id=9606 Gene_Symbol=KRT2 Keratin, type II cytoskeletal 2 epidermal	CON__P35908;CON__Q7Z794;tr G3X8G9 G3X8G9_BOVIN;sp P05786 K2C8_BOVIN;CON__Q9H552;CON__Q9R0H5;tr G3MYU2 G3MYU2_BOVIN;sp P04262 K2CB_BOVIN;sp P04261 K2C3_BOVIN;sp P04260 K2C4_BOVIN;tr A4IFN4 A4IFN4_BOVIN;tr E1BIX9 E1BIX9_BOVIN;tr F1MU12 F1MU12_BOVIN;CON__Q14CN4-1;CON__Q6IME9;CON__Q3SY84;sp Q148H8 K2C72_BOVIN;sp Q148H5 K2C71_BOVIN;CON__Q7RTS7;CON__Q32MB2;sp A3KN27 K2C74_BOVIN;tr E1B991 E1B991_BOVIN;tr G3MZ71 G3MZ71_BOVIN	27.001	NaN	EV fraction only	4.50E+06
	General vesicular transport factor p115	27.009	NaN	EV fraction only	3.10E+07
	Histone H4 (Fragment)	27.033	NaN	EV fraction only	4.10E+07
Uncharacterized protein	tr F1MZX2 F1MZX2_BOVIN	27.040	NaN	EV fraction only	9.70E+06

Signal transducer and activator of transcription	tr B0JYL6 B0JYL6_BOVIN	27.060	NaN	EV fraction only	4.60E+06
Uncharacterized protein	tr E1BPK6 E1BPK6_BOVIN	27.067	NaN	EV fraction only	3.40E+06
Uncharacterized protein (Fragment)	tr E1BB36 E1BB36_BOVIN	27.135	NaN	EV fraction only	8.70E+06
Aspartate--tRNA ligase, cytoplasmic	tr F1MTX7 F1MTX7_BOVIN	27.282	NaN	EV fraction only	5.30E+06
Uncharacterized protein	tr F1MBF6 F1MBF6_BOVIN;tr G3X7D3 G3X7D3_BOVIN;tr F1MX39 F1MX39_BOVIN	27.330	NaN	EV fraction only	4.60E+07
Sodium/potassium-transporting ATPase subunit alpha-1	tr F1MI32 F1MI32_BOVIN;sp Q08DA1 AT1A1_BOVIN;sp A2VDL6 AT1A2_BOVIN;tr F1MR06 F1MR06_BOVIN;tr E1B8N5 E1B8N5_BOVIN;tr F1N1K4 F1N1K4_BOVIN;tr G3N2I3 G3N2I3_BOVIN;tr F1MXW4 F1MXW4_BOVIN	27.405	NaN	EV fraction only	3.90E+06
Uncharacterized protein	tr F1MIF2 F1MIF2_BOVIN	27.572	NaN	EV fraction only	4.50E+06
Tubulin beta-2B chain	sp Q6B856 TBB2B_BOVIN;tr G3N1W7 G3N1W7_BOVIN	27.788	NaN	EV fraction only	1.20E+07
Microfibrillar-associated protein 2	tr F1MWK6 F1MWK6_BOVIN;sp P27424 MFAP2_BOVIN	28.384	NaN	EV fraction only	4.00E+08
Cystatin-C	sp P01035 CYTC_BOVIN	28.772	NaN	EV fraction only	1.20E+08
Estradiol 17-beta-dehydrogenase 12	tr A6H7H3 A6H7H3_BOVIN	29.508	NaN	EV fraction only	4.80E+07

Uncharacterized protein	tr E1BDX8 E1BDX8_BOVIN	30.705	NaN	EV fraction only	6.90E+06
Protein FAM188A	sp Q0IIH8 F188A_BOVIN	NaN	19.040	Total	4.80E+05
Coagulation factor V	tr F1N0I3 F1N0I3_BOVIN;sp Q28107 FA5_BOVIN;CON_Q28107	NaN	19.050	Total	9.30E+04
Fructose-2,6-bisphosphatase TIGAR	sp Q1JQA7 TIGAR_BOVIN	NaN	19.685	Total	8.10E+05
Non-histone chromosomal protein HMG-17	tr F2Z4H2 F2Z4H2_BOVIN;sp P02313 HMG2_BOVIN	NaN	19.875	Total	6.50E+06
Heterochromatin protein 1-binding protein 3	sp Q08DU9 HP1B3_BOVIN	NaN	20.120	Total	7.70E+05
Uncharacterized protein	tr E1B9Y0 E1B9Y0_BOVIN	NaN	20.142	Total	6.20E+05
Protein pelota homolog	sp Q58DV0 PELO_BOVIN	NaN	20.144	Total	7.40E+05
Eukaryotic initiation factor 4A-III	sp Q2NL22 IF4A3_BOVIN	NaN	20.172	Total	9.40E+05
Calpain small subunit 1	sp P13135 CPNS1_BOVIN	NaN	20.247	Total	1.50E+06
Calsequestrin	tr Q3MHM1 Q3MHM1_BOVIN	NaN	20.279	Total	1.00E+06
Protein mago nashi homolog	sp Q3ZBV3 MGN_BOVIN;sp Q0VC92 MGN2_BOVIN	NaN	20.303	Total	1.70E+06
TNFRSF6B protein	tr A6QPW7 A6QPW7_BOVIN	NaN	20.341	Total	1.80E+06
Uncharacterized protein	tr E1BMN6 E1BMN6_BOVIN	NaN	20.347	Total	6.20E+05

Uncharacterized protein	tr E1BJW3 E1BJW3_BOVIN	NaN	20.404	Total vitreous	4.30E+05
Uncharacterized protein	tr F1MIZ7 F1MIZ7_BOVIN	NaN	20.453	Total vitreous	6.70E+05
U6 snRNA-associated Sm-like protein LSm4	sp Q3ZBK6 LSM4_BOVIN	NaN	20.459	Total vitreous	2.20E+06
DnaJ homolog subfamily A member 1	sp Q5E954 DNJA1_BOVIN;tr A6QM13 A6QM13_BOVIN	NaN	20.497	Total vitreous	1.00E+06
Uncharacterized protein	tr E1BIG6 E1BIG6_BOVIN	NaN	20.501	Total vitreous	6.20E+05
15 kDa selenoprotein	sp A8YXY3 SEP15_BOVIN	NaN	20.564	Total vitreous	2.80E+06
FAM151B protein	tr A5PKK0 A5PKK0_BOVIN	NaN	20.600	Total vitreous	1.80E+06
Uncharacterized protein	tr F1MBQ8 F1MBQ8_BOVIN	NaN	20.626	Total vitreous	6.80E+05
PC4 and SFRS1-interacting protein	tr E1BP00 E1BP00_BOVIN;sp Q8MJG1 PSIP1_BOVIN	NaN	20.661	Total vitreous	7.20E+05
Uncharacterized protein (Fragment)	tr F1N3R6 F1N3R6_BOVIN	NaN	20.729	Total vitreous	6.00E+05
Septin-8	sp Q0VCP4 SEPT8_BOVIN;tr F1MDV8 F1MDV8_BOVIN;sp Q2KJB1 SEP10_BOVIN	NaN	20.732	Total vitreous	1.40E+06
Uncharacterized protein	tr F1MHX0 F1MHX0_BOVIN	NaN	20.784	Total vitreous	6.10E+05
Costars family protein ABRACL	sp Q3ZBN0 ABRAL_BOVIN	NaN	20.795	Total vitreous	4.90E+06
ADAM23 protein	tr A4FUX7 A4FUX7_BOVIN	NaN	20.803	Total vitreous	7.10E+05

Cholinesterase	sp P32749 CHLE_BOVIN	NaN	20.809	Total	8.80E+05
Uncharacterized protein (Fragment)	tr F1MG74 F1MG74_BOVIN	NaN	20.840	Total	1.20E+06
Phosphorylase	tr F1MJ28 F1MJ28_BOVIN;sp P79334 PYGM_BOVIN	NaN	20.874	Total	7.40E+05
CRISPLD1 protein	tr A6QR60 A6QR60_BOVIN	NaN	20.886	Total	1.00E+06
Adenylosuccinate synthetase isozyme 2	sp A7MBG0 PURA2_BOVIN	NaN	20.926	Total	1.30E+06
Uncharacterized protein	tr E1BIS6 E1BIS6_BOVIN	NaN	20.968	Total	3.30E+05
Uncharacterized protein (Fragment)	tr F1N0C0 F1N0C0_BOVIN	NaN	20.980	Total	4.60E+05
Uncharacterized protein	tr F1MU85 F1MU85_BOVIN	NaN	20.992	Total	1.70E+06
Ubiquitin-like protein 5	tr G3MY86 G3MY86_BOVIN;sp Q3T0Z3 UBL5_BOVIN	NaN	21.003	Total	7.10E+06
Metallo-beta-lactamase domain-containing protein 1	tr F1MBD2 F1MBD2_BOVIN;sp Q2HJB0 MBLC1_BOVIN	NaN	21.006	Total	2.60E+06
Uncharacterized protein	tr G3X7R5 G3X7R5_BOVIN;tr F1MBI6 F1MBI6_BOVIN;tr G3N2N4 G3N2N4_BOVIN;tr F1MS84 F1MS84_BOVIN	NaN	21.007	Total	1.50E+06
Uncharacterized protein (Fragment)	tr F1MZD0 F1MZD0_BOVIN	NaN	21.013	Total	2.30E+05
Uncharacterized protein	tr E1BL29 E1BL29_BOVIN	NaN	21.026	Total	1.10E+06

CHMP4B protein	tr Q08E32 Q08E32_BOVIN	NaN		21.035	Total	2.10E+06
UBX domain-containing protein 1	sp Q32KW2 UBXN1_BOVIN	NaN		21.040	Total	1.90E+06
Cathepsin S	sp P25326 CATS_BOVIN	NaN		21.193	Total	2.50E+06
Signal-regulatory protein delta	tr Q2TA28 Q2TA28_BOVIN	NaN		21.207	Total	2.20E+06
Ecdysoless homolog (Drosophila)	tr Q2KI67 Q2KI67_BOVIN	NaN		21.234	Total	1.00E+06
Uncharacterized protein (Fragment)	tr F1MC13 F1MC13_BOVIN	NaN		21.243	Total	2.00E+05
Uncharacterized protein	tr E1BPZ4 E1BPZ4_BOVIN	NaN		21.267	Total	5.70E+06
NHL repeat-containing protein 2	sp A4IF69 NHLC2_BOVIN	NaN		21.281	Total	1.40E+06
Lambda-crystallin homolog	sp Q8SPX7 CRYL1_BOVIN	NaN		21.296	Total	2.10E+06
Complexin-1	sp Q0IIL7 CPLX1_BOVIN	NaN		21.304	Total	7.00E+06
Uncharacterized protein (Fragment)	tr F1MZ93 F1MZ93_BOVIN	NaN		21.308	Total	9.70E+05
Transcription elongation factor A protein 1 (Fragment)	tr F1MIT2 F1MIT2_BOVIN;sp Q29RL9 TCEA1_BOVIN;sp Q148K0 TCEA2_BOVIN;tr G1K224 G1K224_BOVIN	NaN		21.313	Total	2.20E+06
FBXO22 protein	tr A5PJX0 A5PJX0_BOVIN	NaN		21.319	Total	2.10E+06

Uncharacterized protein	tr F1MIX9 F1MIX9_BOVIN	NaN	21.322	Total	8.80E+05
Uncharacterized protein (Fragment)	tr F1MFY9 F1MFY9_BOVIN	NaN	21.328	Total	2.20E+06
Regakine-1	sp P82943 REG1_BOVIN	NaN	21.358	Total	6.00E+06
CHN1 protein	tr A7Z037 A7Z037_BOVIN;tr Q0VD41 Q0VD41_BOVIN	NaN	21.367	Total	1.50E+06
Uncharacterized protein	tr E1BGJ4 E1BGJ4_BOVIN	NaN	21.372	Total	7.00E+05
Uncharacterized protein (Fragment)	tr F1MFI5 F1MFI5_BOVIN	NaN	21.388	Total	4.70E+05
Iron-sulfur cluster scaffold homolog (E. coli)	tr Q17QE6 Q17QE6_BOVIN	NaN	21.403	Total	3.10E+06
Splicing factor 1	tr A2VDM7 A2VDM7_BOVIN	NaN	21.424	Total	2.40E+06
Uncharacterized protein	tr F1MG94 F1MG94_BOVIN;tr A7Z085 A7Z085_BOVIN	NaN	21.429	Total	2.60E+06
Polyadenylate-binding protein 1	sp P61286 PABP1_BOVIN;tr A4IFC3 A4IFC3_BOVIN	NaN	21.441	Total	1.10E+06
Alanyl-tRNA-editing protein Aarsd1	tr F1MZR1 F1MZR1_BOVIN;sp Q32LK1 AASD1_BOVIN;tr E1BP20 E1BP20_BOVIN	NaN	21.453	Total	1.70E+06
Uncharacterized protein	tr F1N761 F1N761_BOVIN	NaN	21.491	Total	2.70E+06
PKIB protein	tr Q0VCK2 Q0VCK2_BOVIN	NaN	21.521	Total	1.00E+07
WD repeat-containing protein 44	tr G3X7E3 G3X7E3_BOVIN;sp Q9XSC3 WDR44_BOVIN	NaN	21.539	Total	7.80E+05

Rap1 GTPase-GDP dissociation stimulator 1	sp Q04173 GDS1_BOVIN	NaN	21.539	Total vitreous	1.50E+06
Uncharacterized protein (Fragment)	tr F1MDD8 F1MDD8_BOVIN	NaN	21.544	Total vitreous	1.80E+06
Transforming growth factor-beta-induced protein ig-h3 (Fragment)	tr F1MBS3 F1MBS3_BOVIN	NaN	21.564	Total vitreous	1.30E+06
Phytanoyl-CoA hydroxylase-interacting protein	sp Q0VD34 PHYIP_BOVIN	NaN	21.577	Total vitreous	2.60E+06
Ubiquitin carboxyl-terminal hydrolase	tr G5E630 G5E630_BOVIN	NaN	21.594	Total vitreous	3.80E+05
Selenide, water dikinase 1	sp Q0VC82 SPS1_BOVIN	NaN	21.598	Total vitreous	3.80E+06
Collagen alpha-1(IV) chain (Fragment)	sp Q7SIB2 CO4A1_BOVIN;tr G1K238 G1K238_BOVIN	NaN	21.604	Total vitreous	4.80E+06
Uncharacterized protein (Fragment)	tr G3N286 G3N286_BOVIN	NaN	21.606	Total vitreous	1.10E+07
Chitinase-3-like protein 1	sp P30922 CH3L1_BOVIN;tr G3X7D2 G3X7D2_BOVIN	NaN	21.609	Total vitreous	2.10E+06
FAS-associated death domain protein	sp Q645M6 FADD_BOVIN	NaN	21.614	Total vitreous	3.60E+06
Uncharacterized protein	tr F1N4Y5 F1N4Y5_BOVIN	NaN	21.617	Total vitreous	2.30E+06
Epsilon-sarcoglycan	tr F1MYZ0 F1MYZ0_BOVIN;sp Q29S03 SGCE_BOVIN	NaN	21.618	Total vitreous	2.20E+06
Protein dpy-30 homolog	sp Q2NKU6 DPY30_BOVIN	NaN	21.625	Total vitreous	1.10E+07
Adenosine deaminase-like protein	tr F1N1T1 F1N1T1_BOVIN;sp Q0VC13 ADAL_BOVIN	NaN	21.632	Total vitreous	2.60E+06

Cytosol aminopeptidase	tr G3N0I4 G3N0I4_BOVIN;sp P00727-2 AMPL_BOVIN;sp P00727 AMPL_BOVIN	NaN	21.638	Total vitreous	1.60E+06
Uncharacterized protein	tr F1N7G0 F1N7G0_BOVIN	NaN	21.659	Total vitreous	1.90E+06
Calpastatin	tr G3N2N7 G3N2N7_BOVIN;tr F1MD74 F1MD74_BOVIN;sp P20811 ICAL_BOVIN;tr F1N161 F1N161_BOVIN;tr F1MR96 F1MR96_BOVIN	NaN	21.663	Total vitreous	2.20E+06
Leucine zipper transcription factor-like protein 1 (Fragment)	tr F1MNG7 F1MNG7_BOVIN;sp Q3ZBL4 LZTL1_BOVIN	NaN	21.667	Total vitreous	3.60E+06
MARCKS-related protein	tr G3MZA0 G3MZA0_BOVIN;sp Q0VBZ9 MRP_BOVIN;tr G3MY11 G3MY11_BOVIN	NaN	21.683	Total vitreous	6.50E+06
Uncharacterized protein	tr F1MX44 F1MX44_BOVIN;tr E1BGC0 E1BGC0_BOVIN;sp Q28035 GSTA1_BOVIN;sp O18879 GSTA2_BOVIN	NaN	21.692	Total vitreous	4.20E+06
Uncharacterized protein	tr F1N1F8 F1N1F8_BOVIN	NaN	21.692	Total vitreous	2.40E+05
Carbonyl reductase 3	tr Q0VC97 Q0VC97_BOVIN	NaN	21.697	Total vitreous	3.10E+06
Uncharacterized protein (Fragment)	tr F1N5W4 F1N5W4_BOVIN	NaN	21.708	Total vitreous	3.10E+06
Aspartate aminotransferase, mitochondrial	sp P12344 AATM_BOVIN	NaN	21.715	Total vitreous	1.80E+06

Integrin-linked kinase-associated serine/threonine phosphatase 2C	sp Q0IIF0 ILKAP_BOVIN	NaN	21.723	Total vitreous	2.20E+06
Uroporphyrinogen decarboxylase	tr E1BEX4 E1BEX4_BOVIN	NaN	21.723	Total vitreous	3.10E+06
Heterogeneous nuclear ribonucleoprotein A/B	tr Q3ZC44 Q3ZC44_BOVIN	NaN	21.734	Total vitreous	3.60E+06
ARSB protein	tr A6QLZ3 A6QLZ3_BOVIN	NaN	21.751	Total vitreous	1.70E+06
Uncharacterized protein	tr E1BCU8 E1BCU8_BOVIN	NaN	21.755	Total vitreous	3.20E+06
Uncharacterized protein	tr E1BGE9 E1BGE9_BOVIN	NaN	21.803	Total vitreous	4.50E+06
Uncharacterized protein	tr E1BKX1 E1BKX1_BOVIN	NaN	21.817	Total vitreous	7.50E+05
Uncharacterized protein	tr F6PZ08 F6PZ08_BOVIN	NaN	21.838	Total vitreous	4.20E+06
Uncharacterized protein	tr F1N1A3 F1N1A3_BOVIN;tr Q95M59 Q95M59_BOVIN	NaN	21.849	Total vitreous	3.90E+06
Desmocollin-3 (Fragment)	tr F1N5L6 F1N5L6_BOVIN;sp Q28060-2 DSC3_BOVIN;tr E1BB21 E1BB21_BOVIN;tr F1MXJ3 F1MXJ3_BOVIN;sp Q28060 DSC3_BOVIN	NaN	21.851	Total vitreous	1.10E+06
	N			Total vitreous	3.10E+06
	tr F1MB84 F1MB84_BOVIN			Total vitreous	5.80E+06
	sp Q3T0V7 EDF1_BOVIN			Total vitreous	

Xaa-Pro aminopeptidase 1	sp Q1JPJ2 XPP1_BOVIN	NaN	21.901	Total vitreous	1.60E+06
Follistatin-related protein 3	sp Q1LZB9 FSTL3_BOVIN	NaN	21.904	Total vitreous	4.80E+06
Catalase	sp P00432 CATA_BOVIN	NaN	21.913	Total vitreous	1.80E+06
Calcium regulated heat stable protein 1, 24kDa	tr Q2NКУ4 Q2NКУ4_BOVIN	NaN	21.921	Total vitreous	7.70E+06
Semaphorin-4A	tr F1MY79 F1MY79_BOVIN;sp Q5EA85 SEM4A_BOVIN	NaN	21.922	Total vitreous	1.90E+06
Tissue specific transplantation antigen P35B	tr Q2KIT8 Q2KIT8_BOVIN	NaN	21.937	Total vitreous	3.60E+06
Craniofacial development protein 2	tr F1MS40 F1MS40_BOVIN;sp O02751 CFDP2_BOVIN	NaN	21.955	Total vitreous	1.50E+06
Eukaryotic translation initiation factor 6	sp Q9TU47 IF6_BOVIN	NaN	21.967	Total vitreous	6.10E+06
Vacuolar protein sorting-associated protein 4B	sp Q0VD48 VPS4B_BOVIN	NaN	21.969	Total vitreous	2.30E+06
N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	sp Q3SX44 DDAH2_BOVIN	NaN	21.969	Total vitreous	3.30E+06
Uncharacterized protein (Fragment)	tr E1BKZ1 E1BKZ1_BOVIN	NaN	21.969	Total vitreous	3.10E+06
Uncharacterized protein (Fragment)	tr F1N048 F1N048_BOVIN	NaN	21.979	Total vitreous	2.10E+06
Nascent polypeptide-associated complex subunit alpha	sp Q5E9A1 NACA_BOVIN	NaN	21.997	Total vitreous	9.40E+06

Malignant T-cell-amplified sequence	tr A6QLG1 A6QLG1_BOVIN;sp Q2KIE4 MCTS1_BOVIN	NaN	21.997	Total vitreous	5.60E+06
Alpha-2,8-sialyltransferase ST8Sia II	tr A2BCP4 A2BCP4_BOVIN	NaN	22.004	Total vitreous	2.80E+06
Homeodomain-only protein	sp Q8MJD5 HOP_BOVIN	NaN	22.006	Total vitreous	1.90E+07
Uncharacterized protein	tr F1MXN8 F1MXN8_BOVIN	NaN	22.007	Total vitreous	3.30E+06
Non-histone chromosomal protein HMG-14	sp P02316 HMGN1_BOVIN	NaN	22.015	Total vitreous	1.40E+07
Uncharacterized protein	tr F1MVS4 F1MVS4_BOVIN	NaN	22.015	Total vitreous	6.40E+06
Uncharacterized protein (Fragment)	tr E1BJV1 E1BJV1_BOVIN	NaN	22.059	Total vitreous	2.90E+05
ATP-citrate synthase	sp Q32PF2 ACLY_BOVIN	NaN	22.059	Total vitreous	1.50E+06
Selenium-binding protein 1	sp Q2KJ32 SBP1_BOVIN	NaN	22.060	Total vitreous	1.90E+06
Uncharacterized protein (Fragment)	tr F1MYC4 F1MYC4_BOVIN;sp O62829 PPM1A_BOVIN	NaN	22.066	Total vitreous	3.50E+06
78 kDa glucose-regulated protein	sp Q0VCX2 GRP78_BOVIN;tr F1N614 F1N614_BOVIN	NaN	22.071	Total vitreous	2.10E+06
Alpha-aminoacidipic semialdehyde dehydrogenase	tr E1BFG0 E1BFG0_BOVIN;sp Q2KJC9 AL7A1_BOVIN	NaN	22.088	Total vitreous	2.40E+06
Poly(ADP-ribose) glycohydrolase ARH3	tr F1MWJ3 F1MWJ3_BOVIN;sp Q3SYV9 ARHL2_BOVIN	NaN	22.100	Total vitreous	4.00E+06
LOX protein	tr A5D7S7 A5D7S7_BOVIN;sp P33072 LYOX_BOVIN	NaN	22.109	Total vitreous	3.10E+06

Proteasome subunit beta type-4	sp Q3T108 PSB4_BOVIN	NaN	22.127	Total vitreous	5.60E+06
Glutamate decarboxylase 1	sp Q0VCA1 DCE1_BOVIN	NaN	22.132	Total vitreous	2.60E+06
Uncharacterized protein	tr E1BHY6 E1BHY6_BOVIN	NaN	22.140	Total vitreous	2.50E+06
TOM1L2 protein	tr A5PK10 A5PK10_BOVIN	NaN	22.144	Total vitreous	3.90E+06
Uncharacterized protein	tr E1BIU8 E1BIU8_BOVIN	NaN	22.159	Total vitreous	7.50E+05
Pro-cathepsin H	sp Q3T0I2 CATH_BOVIN	NaN	22.167	Total vitreous	3.70E+06
A disintegrin and metalloproteinase with thrombospondin motifs 5	tr F1MRZ2 F1MRZ2_BOVIN	NaN	22.171	Total vitreous	1.10E+06
NmrA-like family domain-containing protein 1	sp Q0VCN1 NMRL1_BOVIN	NaN	22.178	Total vitreous	4.20E+06
Uncharacterized protein	tr F1MHF7 F1MHF7_BOVIN	NaN	22.186	Total vitreous	9.20E+06
Mitochondrial fission 1 protein	sp Q3T0I5 FIS1_BOVIN	NaN	22.209	Total vitreous	1.00E+07
Proteasome activator complex subunit 2	sp Q5E9G3 PSME2_BOVIN;tr F1MU19 F1MU19_BOVIN	NaN	22.212	Total vitreous	5.40E+06
F-actin-capping protein subunit alpha-1	sp A4FUA8 CAZA1_BOVIN	NaN	22.221	Total vitreous	5.10E+06
Proteasome subunit alpha type-2	sp Q3T0Y5 PSA2_BOVIN	NaN	22.238	Total vitreous	5.10E+06
Uncharacterized protein (Fragment)	tr F1N4I2 F1N4I2_BOVIN;tr Q08DL9 Q08DL9_BOVIN	NaN	22.246	Total vitreous	4.40E+06

GFRA1 protein	tr A7YY41 A7YY41_BOVIN	NaN	22.258	Total vitreous	3.40E+06
Uncharacterized protein	tr E1BKZ9 E1BKZ9_BOVIN	NaN	22.263	Total vitreous	2.10E+06
Cystatin-B	sp P25417 CYTB_BOVIN	NaN	22.266	Total vitreous	1.10E+07
Uncharacterized protein	tr E1B9D4 E1B9D4_BOVIN;tr A2VDV0 A2VDV0_BOVIN	NaN	22.271	Total vitreous	7.90E+06
Uncharacterized protein	tr E1BE14 E1BE14_BOVIN	NaN	22.277	Total vitreous	6.90E+06
Protein phosphatase 1G	sp P79126 PPM1G_BOVIN	NaN	22.279	Total vitreous	3.00E+06
Superoxide dismutase [Mn], mitochondrial	sp P41976 SODM_BOVIN	NaN	22.280	Total vitreous	8.60E+06
Target of myb1	tr Q5BIP4 Q5BIP4_BOVIN	NaN	22.285	Total vitreous	3.30E+06
Uncharacterized protein (Fragment)	tr E1BAK6 E1BAK6_BOVIN	NaN	22.295	Total vitreous	7.70E+06
Prefoldin subunit 2	sp A1A4P5 PFD2_BOVIN	NaN	22.295	Total vitreous	6.90E+06
Uncharacterized protein	tr E1BBU4 E1BBU4_BOVIN	NaN	22.299	Total vitreous	2.00E+06
Harmonin (Fragment)	tr E1BK26 E1BK26_BOVIN;tr F1MIQ5 F1MIQ5_BOVIN;sp Q3MHQ0 USH1C_BOVIN	NaN	22.308	Total vitreous	1.20E+06
Beta-enolase	sp Q3ZC09 ENOB_BOVIN	NaN	22.310	Total vitreous	3.50E+06
Hornerin	CON_Q86YZ3	NaN	22.313	Total vitreous	1.10E+06

Uncharacterized protein (Fragment)	tr E1B8G8 E1B8G8_BOVIN;tr G3N3S9 G3N3S9_BOVIN	NaN	22.322	Total vitreous	1.20E+06
Protein phosphatase 1B	sp O62830 PPM1B_BOVIN	NaN	22.324	Total vitreous	3.10E+06
Dihydrofolate reductase	sp P00376 DYSR_BOVIN	NaN	22.324	Total vitreous	5.90E+06
Uncharacterized protein (Fragment)	tr F1MX14 F1MX14_BOVIN	NaN	22.342	Total vitreous	1.50E+06
Uncharacterized protein	tr F1MBV2 F1MBV2_BOVIN	NaN	22.346	Total vitreous	5.70E+06
Uncharacterized protein	tr E1BDI4 E1BDI4_BOVIN	NaN	22.349	Total vitreous	9.50E+05
3-phosphoadenosine 5-phosphosulfate synthase 1	tr Q3T0J0 Q3T0J0_BOVIN	NaN	22.353	Total vitreous	2.30E+06
Uncharacterized protein	tr E1BPX9 E1BPX9_BOVIN	NaN	22.392	Total vitreous	8.20E+05
Uncharacterized protein (Fragment)	tr G3N019 G3N019_BOVIN	NaN	22.403	Total vitreous	1.40E+06
Growth factor receptor-bound protein 2	tr Q3T0F9 Q3T0F9_BOVIN	NaN	22.419	Total vitreous	5.40E+06
Density-regulated protein	sp Q2HJ47 DENR_BOVIN;tr E1BIK7 E1BIK7_BOVIN	NaN	22.428	Total vitreous	8.50E+06
Probable tRNA N6-adenosine threonylcarbamoyltransferase	sp Q0VC11 OSGEP_BOVIN	NaN	22.429	Total vitreous	5.10E+06
Uncharacterized protein	tr F1MLU5 F1MLU5_BOVIN	NaN	22.440	Total vitreous	1.20E+06

similar to Pregnancy zone protein, partial	CON__ENSEMBL:ENSBTAP00000037665	NaN	22.440	Total vitreous	2.70E+06
Uncharacterized protein	tr F1N611 F1N611_BOVIN	NaN	22.447	Total vitreous	1.80E+06
Uncharacterized protein (Fragment)	tr E1BEI7 E1BEI7_BOVIN	NaN	22.451	Total vitreous	3.90E+06
Ras-related protein Rab-5C	sp Q58DS9 RAB5C_BOVIN	NaN	22.455	Total vitreous	7.90E+06
Cathepsin Z	tr F1MW68 F1MW68_BOVIN;sp P05689 CATZ_BOVIN	NaN	22.461	Total vitreous	7.10E+06
Neuropeptide-like protein C4orf48 homolog	sp A0JNN8 CD048_BOVIN	NaN	22.466	Total vitreous	1.10E+07
Fibroblast growth factor receptor	tr A4IFL5 A4IFL5_BOVIN	NaN	22.477	Total vitreous	2.30E+06
Sialyltransferase 4A	tr A5D960 A5D960_BOVIN	NaN	22.496	Total vitreous	4.40E+06
26S proteasome non-ATPase regulatory subunit 2	sp P56701 PSMD2_BOVIN	NaN	22.499	Total vitreous	1.60E+06
Uncharacterized protein (Fragment)	tr F1MZC0 F1MZC0_BOVIN;tr F1N614 F1N614_BOVIN	NaN	22.499	Total vitreous	7.50E+06
Uncharacterized protein	tr E1BIT8 E1BIT8_BOVIN	NaN	22.502	Total vitreous	2.50E+06
Protein disulfide-isomerase	tr A6H7J6 A6H7J6_BOVIN;sp P05307 PDIA1_BOVIN	NaN	22.518	Total vitreous	2.50E+06
U6 snRNA-associated Sm-like protein LSm3	sp Q32PE9 LSM3_BOVIN	NaN	22.525	Total vitreous	2.70E+07
GATS-like protein 3	sp Q0V8A3 GATL3_BOVIN	NaN	22.532	Total vitreous	8.20E+06

Translation machinery-associated protein 7	sp A1A4Q4 TMA7_BOVIN	NaN	22.542	Total vitreous	4.10E+07
Nucleobindin 2	tr Q0IIH5 Q0IIH5_BOVIN	NaN	22.549	Total vitreous	3.90E+06
Uncharacterized protein	tr E1BIR7 E1BIR7_BOVIN	NaN	22.550	Total vitreous	6.60E+06
Optineurin	tr Q3ZC32 Q3ZC32_BOVIN	NaN	22.552	Total vitreous	2.50E+06
Uncharacterized protein (Fragment)	tr G3MXA7 G3MXA7_BOVIN	NaN	22.558	Total vitreous	2.80E+07
Hepatocyte growth factor receptor	tr Z4YHD9 Z4YHD9_BOVIN;sp Q769I5 MET_BOVIN	NaN	22.567	Total vitreous	1.20E+06
cAMP-dependent protein kinase type I-alpha regulatory subunit	sp P00514 KAP0_BOVIN	NaN	22.576	Total vitreous	4.70E+06
GSTM1 protein	tr A4IFG0 A4IFG0_BOVIN	NaN	22.585	Total vitreous	5.30E+06
Uncharacterized protein	tr F1MMW5 F1MMW5_BOVIN	NaN	22.587	Total vitreous	1.60E+06
Moesin	sp Q2HJ49 MOES_BOVIN	NaN	22.595	Total vitreous	2.60E+06
mRNA cap guanine-N7 methyltransferase	tr F1MHQ5 F1MHQ5_BOVIN	NaN	22.617	Total vitreous	3.90E+06
Uncharacterized protein	tr F1MBV6 F1MBV6_BOVIN;tr Q3ZCA8 Q3ZCA8_BOVIN;tr E1BE76 E1BE76_BOVIN	NaN	22.631	Total vitreous	1.30E+07
Serine/threonine-protein phosphatase	tr Q2KIC7 Q2KIC7_BOVIN	NaN	22.648	Total vitreous	5.50E+06

Septin-2	sp Q2NKY7 SEPT2_BOVIN;tr E1BKU2 E1BKU2_BOVIN	NaN	22.653	Total vitreous	4.80E+06
CHST10 protein	tr A5D799 A5D799_BOVIN	NaN	22.666	Total vitreous	5.60E+06
Uncharacterized protein	tr G5E5H2 G5E5H2_BOVIN	NaN	22.709	Total vitreous	1.80E+07
Uncharacterized protein	tr E1BFZ0 E1BFZ0_BOVIN;tr E1BIC8 E1BIC8_BOVIN	NaN	22.712	Total vitreous	4.10E+06
Bucentaur-2	tr A0JBZ9 A0JBZ9_BOVIN	NaN	22.726	Total vitreous	2.50E+06
Twisted gastrulation homolog 1 (Drosophila)	tr Q0VD44 Q0VD44_BOVIN	NaN	22.736	Total vitreous	9.40E+06
Aldose 1-epimerase	sp Q5EA79 GALM_BOVIN	NaN	22.746	Total vitreous	5.90E+06
Cation-independent mannose-6-phosphate receptor	tr F1MIE6 F1MIE6_BOVIN;sp P08169 MPRI_BOVIN	NaN	22.776	Total vitreous	7.90E+05
Inositol monophosphatase 3	sp Q2KJ53 IMPA3_BOVIN	NaN	22.778	Total vitreous	7.80E+06
Reticulon (Fragment)	tr E1BP30 E1BP30_BOVIN	NaN	22.801	Total vitreous	3.20E+06
S-methyl-5-thioadenosine phosphorylase	sp Q3MHF7 MTAP_BOVIN;tr H9KUV2 H9KUV2_BOVIN	NaN	22.802	Total vitreous	5.50E+06
Calpain-2 catalytic subunit	sp Q27971 CAN2_BOVIN	NaN	22.804	Total vitreous	3.60E+06
TSC22 domain family protein 1	sp Q3MHL6 T22D1_BOVIN;tr E1BCC2 E1BCC2_BOVIN	NaN	22.805	Total vitreous	1.40E+07
Uncharacterized protein	tr F1MEG3 F1MEG3_BOVIN	NaN	22.808	Total vitreous	6.50E+05

Uncharacterized protein	tr F1MUH4 F1MUH4_BOVIN;tr E1B7G3 E1B7G3_BOVIN	NaN	22.812	Total vitreous	4.70E+06
GDNF family receptor alpha-2	tr F1MF66 F1MF66_BOVIN;sp Q5E9X0 GFRA2_BOVIN	NaN	22.814	Total vitreous	4.50E+06
Uncharacterized protein	tr E1BLR9 E1BLR9_BOVIN	NaN	22.833	Total vitreous	1.90E+06
Acidic leucine-rich nuclear phosphoprotein 32 family member B	sp Q3SZC6 AN32B_BOVIN;tr F1MZ46 F1MZ46_BOVIN	NaN	22.845	Total vitreous	1.00E+07
Inhibin beta A chain	sp P07995 INHBA_BOVIN	NaN	22.849	Total vitreous	4.10E+06
Uncharacterized protein	tr F1MLR4 F1MLR4_BOVIN	NaN	22.850	Total vitreous	6.00E+06
Uncharacterized protein	tr E1BA13 E1BA13_BOVIN;sp P62894 CYC_BOVIN;CON__P62894	NaN	22.854	Total vitreous	1.70E+07
Uncharacterized protein	tr F2Z4H3 F2Z4H3_BOVIN	NaN	22.857	Total vitreous	1.10E+07
Uncharacterized protein	tr E1BMJ0 E1BMJ0_BOVIN	NaN	22.860	Total vitreous	6.10E+06
Lysosomal Pro-X carboxypeptidase	tr F1MAU4 F1MAU4_BOVIN;sp Q2TA14 PCP_BOVIN	NaN	22.868	Total vitreous	5.90E+06
Uncharacterized protein (Fragment)	tr F1MDE4 F1MDE4_BOVIN	NaN	22.870	Total vitreous	5.40E+06
PCDHGC3 protein	tr A5D7F4 A5D7F4_BOVIN	NaN	22.872	Total vitreous	2.30E+06
Complement C1q subcomponent subunit B	sp Q2KIV9 C1QB_BOVIN	NaN	22.874	Total vitreous	7.00E+06

Cellular nucleic acid-binding protein	sp Q3T0Q6 CNBP_BOVIN	NaN	22.876	Total vitreous	8.70E+06
Cadherin 11, type 2, OB-cadherin (Osteoblast)	tr A2VDQ6 A2VDQ6_BOVIN	NaN	22.877	Total vitreous	2.80E+06
Uveal autoantigen with coiled-coil domains and ankyrin repeats protein	tr F1MKQ9 F1MKQ9_BOVIN;sp Q8HYY4 UACA_BOVIN;tr F1N101 F1N101_BOVIN	NaN	22.878	Total vitreous	1.20E+06
Glucosidase 2 subunit beta	sp Q28034 GLU2B_BOVIN	NaN	22.881	Total vitreous	5.50E+06
Uncharacterized protein	tr F1MJU6 F1MJU6_BOVIN	NaN	22.897	Total vitreous	3.40E+06
Peptidyl-prolyl cis-trans isomerase (Fragment)	tr E1B9G4 E1B9G4_BOVIN	NaN	22.902	Total vitreous	9.60E+06
Uncharacterized protein	tr F1MME1 F1MME1_BOVIN	NaN	22.908	Total vitreous	1.70E+06
Uncharacterized protein (Fragment)	tr F1N0M5 F1N0M5_BOVIN	NaN	22.946	Total vitreous	7.80E+06
Prefoldin subunit 1	sp Q3SZE2 PFD1_BOVIN	NaN	22.966	Total vitreous	1.40E+07
Uncharacterized protein	tr F1N0F7 F1N0F7_BOVIN	NaN	22.986	Total vitreous	5.10E+06
T-cell surface protein tactile	tr F1N360 F1N360_BOVIN;sp Q3MHP9 TACT_BOVIN	NaN	22.988	Total vitreous	4.00E+06
Uncharacterized protein	tr E1BEC6 E1BEC6_BOVIN	NaN	22.997	Total vitreous	4.20E+06
Uncharacterized protein	tr F1MW03 F1MW03_BOVIN	NaN	23.001	Total vitreous	1.90E+07
Phosphotriesterase-related protein	sp A6QLJ8 PTER_BOVIN	NaN	23.003	Total vitreous	6.00E+06

Glycylpeptide N-tetradecanoyltransferase	tr F1MZK0 F1MZK0_BOVIN;sp P31717 NMT1_BOVIN;sp Q9N181 NMT2_BOVIN;tr G3N0U1 G3N0U1_BOVIN	NaN	23.004	Total vitreous	5.40E+06
Serine-threonine kinase receptor-associated protein	sp Q5E959 STRAP_BOVIN	NaN	23.009	Total vitreous	5.40E+06
HMT1 hnRNP methyltransferase-like 2 isoform 3	tr Q5E949 Q5E949_BOVIN;tr F1MP07 F1MP07_BOVIN	NaN	23.011	Total vitreous	5.30E+06
NPC1 protein	tr B0JYK2 B0JYK2_BOVIN	NaN	23.015	Total vitreous	3.00E+06
Microtubule-associated proteins 1A/1B light chain 3B	sp O41515 MLP3B_BOVIN	NaN	23.036	Total vitreous	2.90E+07
Paraoxonase 1	tr Q2KIW1 Q2KIW1_BOVIN	NaN	23.049	Total vitreous	6.90E+06
Small acidic protein	sp Q3MHL8 SMAP_BOVIN	NaN	23.055	Total vitreous	2.40E+07
Corticosteroid-binding globulin	sp E1BF81 CBG_BOVIN;CON__ENSEMBL:ENSBTAP000000023402	NaN	23.056	Total vitreous	5.70E+06
Uncharacterized protein	tr F1MPJ7 F1MPJ7_BOVIN	NaN	23.069	Total vitreous	1.50E+07
Uncharacterized protein	tr F1MBT2 F1MBT2_BOVIN;tr G3N1F0 G3N1F0_BOVIN	NaN	23.074	Total vitreous	5.40E+06
Cocaine- and amphetamine-regulated transcript protein	sp Q68RJ9 CART_BOVIN	NaN	23.079	Total vitreous	2.40E+07

UDP-Gal:betaGlcNAc beta 1,4-galactosyltransferase, polypeptide 4	tr Q32LF7 Q32LF7_BOVIN	NaN	23.079	Total vitreous	7.70E+06
Phosphoinositide-3-kinase- interacting protein 1	sp Q1RMT9 P3IP1_BOVIN	NaN	23.083	Total vitreous	1.70E+07
Heme-binding protein 1	sp Q148C9 HEBP1_BOVIN;tr G5 E6G2 G5E6G2_BOVIN	NaN	23.086	Total vitreous	1.00E+07
Carbonic anhydrase-related protein 10	sp A0JN41 CAH10_BOVIN	NaN	23.088	Total vitreous	9.30E+06
ATPase, H+ transporting, lysosomal 13kDa, V1 subunit G2	tr Q0VCV6 Q0VCV6_BOVIN;sp P79251 VATG1_BOVIN	NaN	23.097	Total vitreous	1.70E+07
Uncharacterized protein	tr F1MJM5 F1MJM5_BOVIN;tr F 1N444 F1N444_BOVIN	NaN	23.107	Total vitreous	2.10E+06
Heat shock factor-binding protein 1	sp Q3ZC22 HSBP1_BOVIN	NaN	23.129	Total vitreous	2.50E+07
Uncharacterized protein	tr F1N381 F1N381_BOVIN	NaN	23.147	Total vitreous	3.00E+06
Uncharacterized protein	tr F1MHT1 F1MHT1_BOVIN	NaN	23.161	Total vitreous	1.40E+06
Serine/threonine-protein phosphatase	tr F1N719 F1N719_BOVIN	NaN	23.171	Total vitreous	4.60E+06
Uncharacterized protein	tr F1N4K1 F1N4K1_BOVIN	NaN	23.177	Total vitreous	2.50E+06
Uncharacterized protein	tr E1BDS6 E1BDS6_BOVIN	NaN	23.181	Total vitreous	1.50E+06
Uncharacterized protein	tr F1MC76 F1MC76_BOVIN;sp A 3KMX8 CF211_BOVIN	NaN	23.185	Total vitreous	5.40E+06

Uncharacterized protein (Fragment)	tr G3MXB5 G3MXB5_BOVIN	NaN	23.187	Total	2.60E+07
Uncharacterized protein	tr E1BA44 E1BA44_BOVIN;tr G3MZT8 G3MZT8_BOVIN	NaN	23.192	Total	1.90E+06
Uncharacterized protein (Fragment)	tr F1MHR6 F1MHR6_BOVIN	NaN	23.194	Total	3.90E+06
Uncharacterized protein	tr F1N7G8 F1N7G8_BOVIN	NaN	23.214	Total	1.80E+06
Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	sp O77588 PLOD1_BOVIN;tr G8JKV7 G8JKV7_BOVIN	NaN	23.225	Total	3.30E+06
Thimet oligopeptidase	sp Q1JPJ8 THOP1_BOVIN	NaN	23.238	Total	3.70E+06
Thy-1 cell surface antigen	tr Q3SX33 Q3SX33_BOVIN	NaN	23.245	Total	1.90E+07
Uncharacterized protein (Fragment)	tr G5E5K5 G5E5K5_BOVIN	NaN	23.247	Total	2.20E+07
LSM2 protein	tr A6QQV3 A6QQV3_BOVIN	NaN	23.248	Total	2.30E+07
Uncharacterized protein (Fragment)	tr F1N764 F1N764_BOVIN	NaN	23.255	Total	7.50E+06
U6 snRNA-associated Sm-like protein LSM8	sp Q3ZCE0 LSM8_BOVIN	NaN	23.258	Total	1.90E+07
Beta-hexosaminidase subunit alpha	sp Q0V8R6 HEXA_BOVIN	NaN	23.282	Total	6.40E+06
Cysteine-rich protein 2	sp Q0VFX8 CRIP2_BOVIN	NaN	23.283	Total	1.50E+07
Uncharacterized protein	tr F1N2Y8 F1N2Y8_BOVIN	NaN	23.299	Total	3.10E+06

Proteasome subunit beta type-2	sp Q5E9K0 PSB2_BOVIN	NaN	23.305	Total vitreous	1.40E+07
Melanoma inhibitory activity protein 3 (Fragment)	tr G5E5L5 G5E5L5_BOVIN;sp Q0VC16 MIA3_BOVIN	NaN	23.319	Total vitreous	1.30E+06
Uncharacterized protein	tr E1B7B0 E1B7B0_BOVIN	NaN	23.334	Total vitreous	4.80E+06
Glucosamine-6-phosphate isomerase 1	sp A4FV08 GNPI1_BOVIN;sp Q17QL1 GNPI2_BOVIN	NaN	23.338	Total vitreous	8.90E+06
Uncharacterized protein	tr E1BEG2 E1BEG2_BOVIN	NaN	23.343	Total vitreous	8.10E+06
Peptidyl-prolyl cis-trans isomerase-like 1	sp Q5E992 PPIL1_BOVIN	NaN	23.349	Total vitreous	1.80E+07
Hepatocyte growth factor-regulated tyrosine kinase substrate	sp Q0V8S0 HGS_BOVIN	NaN	23.349	Total vitreous	6.60E+06
Mitochondrial peptide methionine sulfoxide reductase	sp P54149 MSRA_BOVIN	NaN	23.358	Total vitreous	9.70E+06
Syntaxin-1B	sp P61267 STX1B_BOVIN	NaN	23.379	Total vitreous	9.20E+06
Uridine diphosphate glucose pyrophosphatase	sp Q05B60 NUD14_BOVIN	NaN	23.381	Total vitreous	1.30E+07
Prosaposin	sp P26779 SAP_BOVIN;tr F1MXP8 F1MXP8_BOVIN	NaN	23.382	Total vitreous	5.30E+06
Serine protease 23	sp Q1LZE9 PRS23_BOVIN	NaN	23.390	Total vitreous	6.40E+06
Neuron specific gene family member 1	tr Q08DZ1 Q08DZ1_BOVIN	NaN	23.394	Total vitreous	1.50E+07

Gamma-aminobutyric acid receptor-associated protein- like 2	tr F1MFF1 F1MFF1_BOVIN;sp P 60519 GBRL2_BOVIN	NaN	23.395	Total vitreous	2.00E+07
Insulin-like growth factor- binding protein 4	sp Q05716 IBP4_BOVIN	NaN	23.399	Total vitreous	1.20E+07
Uncharacterized protein	tr F1N4K6 F1N4K6_BOVIN	NaN	23.421	Total vitreous	1.60E+06
Prefoldin subunit 3	tr G5E5K1 G5E5K1_BOVIN;sp Q 2TBX2 PFD3_BOVIN	NaN	23.433	Total vitreous	1.00E+07
Uncharacterized protein	tr G3MYZ3 G3MYZ3_BOVIN;CO N_REFSEQ:XP_585019	NaN	23.445	Total vitreous	4.70E+06
Uncharacterized protein (Fragment)	tr F1N3V8 F1N3V8_BOVIN	NaN	23.467	Total vitreous	3.10E+07
3-hydroxybutyrate dehydrogenase type 2	tr F1MLA4 F1MLA4_BOVIN;sp Q 3T046 BDH2_BOVIN	NaN	23.471	Total vitreous	1.30E+07
Glypican-1	sp G3X745 GPC1_BOVIN	NaN	23.483	Total vitreous	5.90E+06
Uncharacterized protein (Fragment)	tr F1MXM6 F1MXM6_BOVIN	NaN	23.518	Total vitreous	1.10E+07
Purkinje cell protein 4-like protein 1	sp A8R4Q8 PC4L1_BOVIN	NaN	23.528	Total vitreous	4.10E+07
Beta-1,3-N- acetylglucosaminyltransfera se lunatic fringe	sp Q2KJ92 LFNG_BOVIN	NaN	23.550	Total vitreous	8.70E+06
Uncharacterized protein	tr E1B8P9 E1B8P9_BOVIN	NaN	23.552	Total vitreous	1.20E+07
Uncharacterized protein	tr F1N3N6 F1N3N6_BOVIN	NaN	23.567	Total vitreous	7.30E+06

Uncharacterized protein (Fragment)	tr G5E5W4 G5E5W4_BOVIN;tr F1MYL5 F1MYL5_BOVIN;tr F1MES2 F1MES2_BOVIN	NaN	23.568	Total vitreous	3.00E+06
Drebrin-like protein	sp A6H7G2 DBNL_BOVIN	NaN	23.586	Total vitreous	8.10E+06
Uncharacterized protein	tr F6PZ29 F6PZ29_BOVIN	NaN	23.587	Total vitreous	2.40E+07
Uncharacterized protein	tr G3N0B6 G3N0B6_BOVIN	NaN	23.604	Total vitreous	6.10E+06
Uncharacterized protein	tr F1N789 F1N789_BOVIN	NaN	23.644	Total vitreous	2.70E+06
Uncharacterized protein (Fragment)	tr F1MXN4 F1MXN4_BOVIN;tr E1BFN8 E1BFN8_BOVIN	NaN	23.645	Total vitreous	8.00E+06
2-deoxynucleoside 5-phosphate N-hydrolase 1	tr E1BM28 E1BM28_BOVIN	NaN	23.656	Total vitreous	2.00E+07
Integral membrane protein 2B	tr F1N026 F1N026_BOVIN;sp Q3T0P7 ITM2B_BOVIN	NaN	23.664	Total vitreous	1.30E+07
Uncharacterized protein (Fragment)	tr F1MC06 F1MC06_BOVIN;tr G3MYW6 G3MYW6_BOVIN	NaN	23.673	Total vitreous	1.60E+07
Uncharacterized protein	tr F1MUE3 F1MUE3_BOVIN	NaN	23.678	Total vitreous	7.90E+06
Proteasome subunit alpha type-4	sp Q3ZCK9 PSA4_BOVIN	NaN	23.681	Total vitreous	1.40E+07
Neural cell adhesion molecule 1 (Fragment)	tr F1N1W7 F1N1W7_BOVIN	NaN	23.683	Total vitreous	5.00E+06
Microtubule-associated proteins 1A/1B light chain 3A	sp Q2HJ23 MLP3A_BOVIN	NaN	23.687	Total vitreous	4.60E+07

Lysyl oxidase homolog 1	sp Q95L39 LOXL1_BOVIN;tr A0JNB6 A0JNB6_BOVIN	NaN	23.690	Total vitreous	6.80E+06
Calcium-binding protein 39	sp Q29RI6 CAB39_BOVIN	NaN	23.690	Total vitreous	1.10E+07
Histidine--tRNA ligase, cytoplasmic	sp Q2KI84 SYHC_BOVIN	NaN	23.696	Total vitreous	6.10E+06
LIM and SH3 domain protein 1	sp Q3B7M5 LASP1_BOVIN	NaN	23.696	Total vitreous	1.30E+07
NAD(P)H dehydrogenase, quinone 2	tr Q3SZT2 Q3SZT2_BOVIN	NaN	23.700	Total vitreous	1.40E+07
Uncharacterized protein	tr F1MET0 F1MET0_BOVIN	NaN	23.705	Total vitreous	4.30E+06
Prefoldin subunit 5	sp Q8HYI9 PFD5_BOVIN	NaN	23.717	Total vitreous	2.40E+07
Collectin-12	sp A6QP79 COL12_BOVIN	NaN	23.739	Total vitreous	5.10E+06
Chromosome 10 open reading frame 116 ortholog	tr Q2NKR5 Q2NKR5_BOVIN	NaN	23.740	Total vitreous	6.30E+07
Dynamin-2	sp A6H7I5 DYN2_BOVIN;tr F1MW91 F1MW91_BOVIN	NaN	23.755	Total vitreous	4.70E+06
RAN binding protein 6	tr Q3B7N3 Q3B7N3_BOVIN	NaN	23.757	Total vitreous	8.30E+06
Uncharacterized protein	tr E1BER5 E1BER5_BOVIN;tr F1MHZ5 F1MHZ5_BOVIN	NaN	23.759	Total vitreous	3.70E+06
Uncharacterized protein	tr G3N3R5 G3N3R5_BOVIN;tr G3MWN4 G3MWN4_BOVIN;tr E1BAB5 E1BAB5_BOVIN	NaN	23.772	Total vitreous	5.90E+06
Alpha-synuclein	sp Q3T0G8 SYUA_BOVIN	NaN	23.786	Total vitreous	3.30E+07

Uncharacterized protein	tr G3N1H5 G3N1H5_BOVIN;tr G3N1R1 G3N1R1_BOVIN;tr G3MWT1 G3MWT1_BOVIN;tr G3N148 G3N148_BOVIN	NaN	23.805	Total vitreous	2.80E+07
Hexokinase 1	tr Q5W5U3 Q5W5U3_BOVIN;tr F1MZV1 F1MZV1_BOVIN;sp P27595 HXK1_BOVIN;tr E1BME6 E1BME6_BOVIN;tr F1MIM3 F1MIM3_BOVIN	NaN	23.808	Total vitreous	4.20E+06
Transketolase	sp Q6B855 TKT_BOVIN	NaN	23.812	Total vitreous	7.40E+06
Uncharacterized protein (Fragment)	tr E1BCE0 E1BCE0_BOVIN	NaN	23.829	Total vitreous	1.70E+07
Translin	sp Q08DM8 TSN_BOVIN	NaN	23.838	Total vitreous	1.80E+07
Fas apoptotic inhibitory molecule 1	sp Q0IIF6 FAIM1_BOVIN	NaN	23.840	Total vitreous	2.00E+07
ST6 (Alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1, 3)-N-acetyl/galactosaminide alpha-2,6-sialyltransferase 2	tr Q148L9 Q148L9_BOVIN	NaN	23.846	Total vitreous	2.10E+07
DUSP3 protein	tr A7YY43 A7YY43_BOVIN;tr Q2T9T7 Q2T9T7_BOVIN	NaN	23.858	Total vitreous	2.70E+07
Secretagogin	tr F1N1I2 F1N1I2_BOVIN;sp A5PJN0 SEGN_BOVIN	NaN	23.859	Total vitreous	1.30E+07
Uncharacterized protein	tr E1BNX1 E1BNX1_BOVIN	NaN	23.865	Total vitreous	2.00E+07

Complement factor properdin	tr Q17QC8 Q17QC8_BOVIN	NaN	23.866	Total vitreous	1.30E+07
Isoform Tau-E of Microtubule-associated protein tau	sp P29172- 5 TAU_BOVIN;sp P29172- 4 TAU_BOVIN;sp P29172- 10 TAU_BOVIN;sp P29172- 16 TAU_BOVIN;sp P29172- 8 TAU_BOVIN;tr G3N2J1 G3N2J 1_BOVIN;sp P29172- 7 TAU_BOVIN;sp P29172- 13 TAU_BOVIN;sp P29172- 12 TAU_BOVIN;sp P29172- 2 TAU_BOVIN;sp P29172- 19 TAU_BOVIN;sp P29172 TAU _BOVIN;sp P29172- 18 TAU_BOVIN;sp P29172- 9 TAU_BOVIN;sp P29172- 15 TAU_BOVIN	NaN	23.887	Total vitreous	1.10E+07
	sp Q17QN8 CL049_BOVIN	NaN	23.889	Total vitreous	2.10E+07
	tr Q17QC7 Q17QC7_BOVIN	NaN	23.898	Total vitreous	8.20E+06
	sp Q148J6 ARPC4_BOVIN	NaN	23.899	Total vitreous	2.70E+07
	tr E1BL59 E1BL59_BOVIN	NaN	23.902	Total vitreous	3.70E+06
	tr F1MIH9 F1MIH9_BOVIN;sp Q 2KIY5 PLBL2_BOVIN	NaN	23.903	Total vitreous	7.30E+06
UPF0454 protein C12orf49 homolog	sp Q17QN8 CL049_BOVIN	NaN	23.889	Total vitreous	2.10E+07
Poliovirus receptor-related 2 (Herpesvirus entry mediator B)	tr Q17QC7 Q17QC7_BOVIN	NaN	23.898	Total vitreous	8.20E+06
Actin-related protein 2/3 complex subunit 4	sp Q148J6 ARPC4_BOVIN	NaN	23.899	Total vitreous	2.70E+07
Uncharacterized protein	tr E1BL59 E1BL59_BOVIN	NaN	23.902	Total vitreous	3.70E+06
Putative phospholipase B- like 2	tr F1MIH9 F1MIH9_BOVIN;sp Q 2KIY5 PLBL2_BOVIN	NaN	23.903	Total vitreous	7.30E+06

Endoplasmic reticulum resident protein 44	sp Q3T0L2 ERP44_BOVIN	NaN	23.904	Total vitreous	1.10E+07
Cysteine and histidine-rich domain-containing protein 1	sp Q29RL2 CHRD1_BOVIN	NaN	23.912	Total vitreous	1.60E+07
Uncharacterized protein	tr F1N0D3 F1N0D3_BOVIN	NaN	23.915	Total vitreous	5.80E+06
Glucose-6-phosphate 1-dehydrogenase	tr F1MMK2 F1MMK2_BOVIN;REV_tr E1BIG2 E1BIG2_BOVIN	NaN	23.918	Total vitreous	6.90E+06
Maturin	sp A7MBJ1 MTURN_BOVIN	NaN	23.920	Total vitreous	7.30E+07
Tyrosine--tRNA ligase, cytoplasmic	tr F1MHM5 F1MHM5_BOVIN;sp Q29465 SYYC_BOVIN	NaN	23.927	Total vitreous	5.80E+06
Uncharacterized protein (Fragment)	tr E1BIB8 E1BIB8_BOVIN	NaN	23.928	Total vitreous	3.40E+07
Coagulation factor IX	tr F1MBC5 F1MBC5_BOVIN;sp P00741 FA9_BOVIN;tr F1MFL4 F1MFL4_BOVIN	NaN	23.934	Total vitreous	1.60E+07
SH3 domain-binding glutamic acid-rich-like protein 2	sp A4IFC4 SH3L2_BOVIN;tr F1MR08 F1MR08_BOVIN	NaN	23.935	Total vitreous	5.50E+07
Uncharacterized protein	tr E1BCA0 E1BCA0_BOVIN	NaN	23.942	Total vitreous	3.50E+06
Peptidase inhibitor 16	sp Q58D34 PI16_BOVIN	NaN	23.952	Total vitreous	9.10E+06
Thymosin beta-10	sp P21752 TYB10_BOVIN;CON_P21752	NaN	24.003	Total vitreous	1.10E+08
Kininogen-1	sp P01044 KNG1_BOVIN;CON_P01044-1;sp P01044-2 KNG1_BOVIN	NaN	24.017	Total vitreous	6.50E+06

Cadherin-13 (Fragment)	tr F1MKP6 F1MKP6_BOVIN;sp Q3B7N0 CAD13_BOVIN	NaN	24.022	Total vitreous	7.70E+06
CD59 molecule, complement regulatory protein	tr Q32PA1 Q32PA1_BOVIN	NaN	24.028	Total vitreous	5.90E+07
Dentin matrix acidic phosphoprotein 1	tr F1MJM1 F1MJM1_BOVIN	NaN	24.028	Total vitreous	8.90E+06
Uncharacterized protein	tr F6S1Q0 F6S1Q0_BOVIN	NaN	24.037	Total vitreous	1.10E+07
Protein S100-A12	sp P79105 S10AC_BOVIN;tr G3N2H5 G3N2H5_BOVIN	NaN	24.043	Total vitreous	3.90E+07
Parvalbumin alpha	sp Q0VCG3 PRVA_BOVIN	NaN	24.044	Total vitreous	2.90E+07
Biotinidase	tr F1MJM4 F1MJM4_BOVIN;sp A6QQ07 BTD_BOVIN	NaN	24.051	Total vitreous	1.10E+07
SCRG1 protein	tr A6QPC2 A6QPC2_BOVIN	NaN	24.054	Total vitreous	3.90E+07
Uncharacterized protein	tr F1MZX8 F1MZX8_BOVIN	NaN	24.063	Total vitreous	8.20E+06
Vitamin K-dependent protein C (Fragment)	sp P00745 PROC_BOVIN	NaN	24.069	Total vitreous	8.30E+06
Proteasome subunit beta type-5	sp Q32KL2 PSB5_BOVIN	NaN	24.086	Total vitreous	1.50E+07
Acyl-CoA-binding domain-containing protein 7	sp Q3SZF0 ACBD7_BOVIN	NaN	24.090	Total vitreous	4.00E+07
Cortactin	tr Q1RMR3 Q1RMR3_BOVIN	NaN	24.097	Total vitreous	8.60E+06
Uncharacterized protein	tr F1ME46 F1ME46_BOVIN	NaN	24.102	Total vitreous	1.20E+07

Procollagen C- endopeptidase enhancer	tr Q2HJB6 Q2HJB6_BOVIN	NaN	24.113	Total vitreous	1.20E+07
Proline synthase co- transcribed bacterial homolog protein	sp Q3T0G5 PROSC_BOVIN	NaN	24.120	Total vitreous	1.80E+07
Putative uncharacterized protein MGC139448	tr A1A4H7 A1A4H7_BOVIN	NaN	24.121	Total vitreous	1.00E+07
Uncharacterized protein	tr E1BJ82 E1BJ82_BOVIN	NaN	24.123	Total vitreous	1.20E+08
Uncharacterized protein (Fragment)	tr F1N104 F1N104_BOVIN;tr G3 X7N7 G3X7N7_BOVIN;tr F6Q4B 1 F6Q4B1_BOVIN	NaN	24.124	Total vitreous	1.10E+07
Uncharacterized protein	tr E1BD36 E1BD36_BOVIN	NaN	24.135	Total vitreous	5.00E+06
Uncharacterized protein	tr F2Z4F5 F2Z4F5_BOVIN	NaN	24.136	Total vitreous	6.50E+06
Uncharacterized protein	tr F1MX87 F1MX87_BOVIN	NaN	24.138	Total vitreous	9.50E+06
Cadherin-3	tr E1BGT1 E1BGT1_BOVIN	NaN	24.150	Total vitreous	7.40E+06
Alpha-amylase	tr F1MJQ3 F1MJQ3_BOVIN;CO N__Q3MHH8;tr F1MP21 F1MP2 1_BOVIN	NaN	24.152	Total vitreous	1.00E+07
Isoamyl acetate-hydrolyzing esterase 1 homolog	sp Q3SZ16 IAH1_BOVIN	NaN	24.155	Total vitreous	2.10E+07
Tripeptidyl-peptidase 1	tr F1MK08 F1MK08_BOVIN;sp Q 0V8B6 TPP1_BOVIN	NaN	24.158	Total vitreous	1.10E+07

Uncharacterized protein	tr E1B7S3 E1B7S3_BOVIN;tr F1MCU7 F1MCU7_BOVIN;sp Q9N179 41_BOVIN;tr F1MCU4 F1MCU4_BOVIN	NaN	24.174	Total vitreous	5.50E+06
Uncharacterized protein (Fragment)	tr E1B805 E1B805_BOVIN	NaN	24.175	Total vitreous	3.10E+06
Chemokine (C-C motif) ligand 14	tr Q29RR9 Q29RR9_BOVIN	NaN	24.175	Total vitreous	4.30E+07
Desmocollin-2	tr F1MZF2 F1MZF2_BOVIN;sp P33545-2 DSC2_BOVIN;sp P33545 DSC2_BOVIN	NaN	24.192	Total vitreous	6.30E+06
Uncharacterized protein (Fragment)	tr F1N6H1 F1N6H1_BOVIN	NaN	24.216	Total vitreous	1.10E+06
Angiopoietin-related protein 7	sp Q5EA66 ANGL7_BOVIN	NaN	24.249	Total vitreous	1.80E+07
Small glutamine-rich tetratricopeptide repeat-containing protein alpha	sp Q32LM2 SGTA_BOVIN	NaN	24.254	Total vitreous	2.10E+07
Pyridoxine-5-phosphate oxidase	sp Q5E9K3 PNPO_BOVIN	NaN	24.254	Total vitreous	2.20E+07
Cathepsin L2	sp Q5E998 CATL2_BOVIN;sp P25975 CATL1_BOVIN;sp Q5E968 CATK_BOVIN;tr A4IFS7 A4IFS7_BOVIN	NaN	24.270	Total vitreous	1.50E+07
Heat shock protein 105 kDa	sp Q0IIM3 HS105_BOVIN	NaN	24.278	Total vitreous	5.80E+06
Glutathione peroxidase 1	sp P00435 GPX1_BOVIN	NaN	24.291	Total vitreous	1.90E+07

Ubiquitin-conjugating enzyme E2 D3	sp Q3ZCF7 UB2D3_BOVIN;sp Q1RMX2 UB2D2_BOVIN;tr G3MZ57 G3MZ57_BOVIN;tr G3MY62 G3MY62_BOVIN;tr G3MXY4 G3MXY4_BOVIN	NaN	24.303	Total vitreous	7.60E+07
Glandular kallikrein	tr Q6H320 Q6H320_BOVIN	NaN	24.317	Total vitreous	3.10E+07
CD166 antigen	tr F1MHN8 F1MHN8_BOVIN;sp Q9BH13 CD166_BOVIN	NaN	24.321	Total vitreous	9.10E+06
Uncharacterized protein (Fragment)	tr F1N554 F1N554_BOVIN;tr F1MLM4 F1MLM4_BOVIN	NaN	24.324	Total vitreous	1.00E+07
Beta-mannosidase	tr A6QLB0 A6QLB0_BOVIN;sp Q29444 MANBA_BOVIN	NaN	24.330	Total vitreous	6.00E+06
Uncharacterized protein (Fragment)	tr G3N2S8 G3N2S8_BOVIN	NaN	24.333	Total vitreous	7.60E+07
Proteasome subunit alpha type	tr G5E5C3 G5E5C3_BOVIN;sp Q2YDE4 PSA6_BOVIN	NaN	24.334	Total vitreous	1.70E+07
Proteasome subunit alpha type-3	sp Q58DU5 PSA3_BOVIN	NaN	24.345	Total vitreous	2.20E+07
Diphosphoinositol polyphosphate phosphohydrolase 1	sp A2VE79 NUDT3_BOVIN	NaN	24.347	Total vitreous	3.20E+07
Uncharacterized protein	tr F6QD94 F6QD94_BOVIN	NaN	24.349	Total vitreous	7.20E+07
FXYD domain-containing ion transport regulator 6	sp Q3MHZ5 FXDYD6_BOVIN	NaN	24.351	Total vitreous	7.20E+07
Adiponectin	sp Q3Y5Z3 ADIPO_BOVIN;CON_Q3Y5Z3	NaN	24.360	Total vitreous	2.30E+07

Uncharacterized protein (Fragment)	tr F1MYZ4 F1MYZ4_BOVIN	NaN	24.365	Total vitreous	7.70E+06
Programmed cell death protein 5	sp Q2HJH9 PDCD5_BOVIN	NaN	24.371	Total vitreous	2.90E+07
Nucleosome assembly protein 1-like 1	sp A6H767 NP1L1_BOVIN;tr G3X7M5 G3X7M5_BOVIN	NaN	24.378	Total vitreous	2.30E+07
Gelsolin	tr F1MJH1 F1MJH1_BOVIN;sp Q3SX14 GELS_BOVIN	NaN	24.387	Total vitreous	9.50E+06
Fatty acid-binding protein, brain	sp Q09139 FABP7_BOVIN	NaN	24.388	Total vitreous	3.70E+07
Phosphatidylinositol-glycan-specific phospholipase D	sp P80109 PHLD_BOVIN	NaN	24.398	Total vitreous	7.90E+06
Uncharacterized protein	tr G1K1Z9 G1K1Z9_BOVIN;sp Q2TA21 CN037_BOVIN	NaN	24.405	Total vitreous	1.50E+07
Protein disulfide-isomerase	tr A5D7E8 A5D7E8_BOVIN;sp P38657 PDIA3_BOVIN	NaN	24.415	Total vitreous	9.70E+06
Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1	sp Q5BIN5 PIN1_BOVIN	NaN	24.420	Total vitreous	3.00E+07
Serotransferrin	tr G3X6N3 G3X6N3_BOVIN	NaN	24.431	Total vitreous	9.50E+06
Uncharacterized protein	tr F1MZK8 F1MZK8_BOVIN	NaN	24.455	Total vitreous	8.20E+06
Plasminogen	sp P06868 PLMN_BOVIN;CON_P06868	NaN	24.472	Total vitreous	6.30E+06
G protein-coupled receptor 37 (Endothelin receptor type B-like)	tr Q0VD43 Q0VD43_BOVIN	NaN	24.482	Total vitreous	1.10E+07

Uncharacterized protein	tr F1N2I5 F1N2I5_BOVIN	NaN		24.490	Total	2.40E+07
Receptor-type tyrosine- protein phosphatase F	sp A7MBJ4 PTPRF_BOVIN	NaN		24.494	Total	3.20E+06
Uncharacterized protein	tr F1N0N6 F1N0N6_BOVIN;sp Q32P66 CA123_BOVIN	NaN		24.501	Total	3.20E+07
Uncharacterized protein	tr F1N678 F1N678_BOVIN	NaN		24.514	Total	1.50E+07
Prothymosin alpha	sp P01252 PTMA_BOVIN	NaN		24.524	Total	1.10E+08
72 kDa type IV collagenase	tr F1MKH8 F1MKH8_BOVIN;sp Q9GLE5 MMP2_BOVIN	NaN		24.532	Total	1.10E+07
Uncharacterized protein (Fragment)	tr F1MCM7 F1MCM7_BOVIN	NaN		24.540	Total	6.60E+06
F-actin-capping protein subunit alpha-2	sp Q5E997 CAZA2_BOVIN	NaN		24.542	Total	2.80E+07
Heterogeneous nuclear ribonucleoproteins A2/B1	sp Q2HJ60 ROA2_BOVIN	NaN		24.547	Total	1.80E+07
Proteasome subunit alpha type-1	sp Q3T0X5 PSA1_BOVIN	NaN		24.575	Total	2.00E+07
121 kDa protein	CON_ENSEMBL:ENSBTAP00000031900	NaN		24.575	Total	6.60E+06
N-acetylglucosamine-6- sulfatase	tr F1MXZ0 F1MXZ0_BOVIN;sp Q1LZH9 GNS_BOVIN	NaN		24.576	Total	1.50E+07
Proteasome subunit alpha type-7	sp Q3ZBG0 PSA7_BOVIN;tr E1BD83 E1BD83_BOVIN	NaN		24.579	Total	2.40E+07
Cochlin	tr F1N103 F1N103_BOVIN;sp Q5EA64 COCH_BOVIN	NaN		24.586	Total	1.20E+07

Inactive serine protease 35	sp Q5E9X7 PR35_BOVIN	NaN	24.591	Total	1.90E+07
Histidine-rich glycoprotein (Fragments)	sp P33433 HRG_BOVIN	NaN	24.628	Total	1.90E+07
Parathymosin	sp P08814 PTMS_BOVIN	NaN	24.639	Total	1.20E+08
DNA damage-binding protein 1	sp A1A4K3 DDB1_BOVIN	NaN	24.640	Total	6.40E+06
Heat shock protein beta-6	sp Q148F8 HSPB6_BOVIN	NaN	24.641	Total	5.00E+07
Uncharacterized protein (Fragment)	tr F1N1R9 F1N1R9_BOVIN	NaN	24.643	Total	1.60E+07
Guanine nucleotide-binding protein G(T) subunit gamma-T1	sp P02698 GBG1_BOVIN	NaN	24.660	Total	1.20E+08
Coactosin-like protein	sp Q2HJ57 COTL1_BOVIN	NaN	24.677	Total	4.00E+07
Prolyl endopeptidase (Fragment)	tr F6QHN4 F6QHN4_BOVIN;sp Q9XTA2 PPCE_BOVIN	NaN	24.681	Total	7.90E+06
Cathepsin D	sp P80209 CATD_BOVIN	NaN	24.683	Total	2.00E+07
Legumain	sp Q95M12 LGMN_BOVIN	NaN	24.688	Total	2.00E+07
Retinol-binding protein 3	tr F1MLW4 F1MLW4_BOVIN	NaN	24.691	Total	6.90E+06
Gamma-aminobutyric acid receptor-associated protein-like 1	sp Q8HYB6 GBRL1_BOVIN;sp Q9GJW7 GBRAP_BOVIN	NaN	24.697	Total	1.20E+08

Ran-specific GTPase-activating protein	sp Q3T0M7 RANG_BOVIN;tr G5E582 G5E582_BOVIN;tr G3MX11 G3MX11_BOVIN	NaN	24.699	Total vitreous	4.60E+07
Serpin A3-2	sp A2I7M9 SPA32_BOVIN	NaN	24.706	Total vitreous	2.10E+07
Uncharacterized protein (Fragment)	tr F1MJR2 F1MJR2_BOVIN	NaN	24.713	Total vitreous	4.20E+06
Ras-related C3 botulinum toxin substrate 1	sp P62998 RAC1_BOVIN;tr F1MNG3 F1MNG3_BOVIN;sp Q9TU25 RAC2_BOVIN;tr F1MCR0 F1MCR0_BOVIN	NaN	24.722	Total vitreous	5.40E+07
Retinoic acid receptor responder protein 2	sp Q29RS5 RARR2_BOVIN	NaN	24.731	Total vitreous	4.70E+07
S-adenosylmethionine synthase	tr A7E3T7 A7E3T7_BOVIN;tr G5E5U7 G5E5U7_BOVIN;sp Q2KJ C6 METK1_BOVIN	NaN	24.757	Total vitreous	2.20E+07
NSFL1 cofactor p47	sp Q3SZC4 NSF1C_BOVIN	NaN	24.767	Total vitreous	1.80E+07
Acetylcholinesterase	sp P23795 ACES_BOVIN;sp P23795-2 ACES_BOVIN;tr F1MIM4 F1MIM4_BOVIN	NaN	24.773	Total vitreous	1.50E+07
Uncharacterized protein	tr E1BL08 E1BL08_BOVIN	NaN	24.777	Total vitreous	8.60E+06
Threonine--tRNA ligase, cytoplasmic	sp Q3ZBV8 SYTC_BOVIN	NaN	24.788	Total vitreous	9.60E+06

Thioredoxin reductase 1, cytoplasmic	tr G1K1Q2 G1K1Q2_BOVIN;sp O62768 TRXR1_BOVIN;tr G3MWU1 G3MWU1_BOVIN;tr F1MN10 F1MN10_BOVIN;sp Q9N2I8 TRXR2_BOVIN	NaN	24.793	Total vitreous	1.60E+07
Calsyntenin-3	sp Q0VCN6 CSTN3_BOVIN	NaN	24.802	Total vitreous	1.10E+07
Tropomyosin alpha-1 chain (Fragment)	tr G3X7S7 G3X7S7_BOVIN;sp Q5KR47-2 TPM3_BOVIN;tr A6QR15 A6QR15_BOVIN;sp Q5KR49 TPM1_BOVIN;sp Q5KR48-2 TPM2_BOVIN;sp Q5KR48 TPM2_BOVIN;sp Q5KR47 TPM3_BOVIN;CON__Q3SX28;tr F1MV90 F1MV90_BOVIN	NaN	24.804	Total vitreous	3.00E+07
	tr A7MBG9 A7MBG9_BOVIN;sp Q1JPH6 JF4H_BOVIN	NaN	24.805	Total vitreous	3.30E+07
	sp P03968 FGF1_BOVIN	NaN	24.808	Total vitreous	5.00E+07
	tr F1MGN0 F1MGN0_BOVIN	NaN	24.810	Total vitreous	9.80E+06
Uncharacterized protein	tr F1N468 F1N468_BOVIN;tr F1MX22 F1MX22_BOVIN	NaN	24.814	Total vitreous	2.30E+07
Putative GTP cyclohydrolase 1 type 2 NIF3L1	sp Q05B89 GTPC1_BOVIN	NaN	24.831	Total vitreous	2.20E+07
Uncharacterized protein	tr F1MLW8 F1MLW8_BOVIN	NaN	24.831	Total vitreous	4.00E+07

High mobility group nucleosome-binding domain-containing protein 3	sp Q3ZBV4 HMGN3_BOVIN	NaN	24.836	Total vitreous	1.40E+08
	tr Q2NKV6 Q2NKV6_BOVIN;tr Q17QH0 Q17QH0_BOVIN;sp P68301 MT2_BOVIN;sp P67983 MT1A_BOVIN;sp P58280 MT1_BOVIN;sp P55943 MT2H_BOVIN;sp P55942 MT1H_BOVIN;CON_P67983	NaN	24.860	Total vitreous	1.40E+08
Metallothionein		NaN	24.873	Total vitreous	1.40E+07
Uncharacterized protein (Fragment)	tr F1MU84 F1MU84_BOVIN	NaN	24.928	Total vitreous	4.80E+07
Mth938 domain-containing protein	sp Q32PA8 AAMDC_BOVIN	NaN	24.934	Total vitreous	8.30E+06
Complement component C6	tr F1MM86 F1MM86_BOVIN;sp Q29RU4 CO6_BOVIN	NaN	24.971	Total vitreous	3.10E+07
Serpin A3-6	sp A2I7N2 SPA36_BOVIN	NaN	24.988	Total vitreous	9.10E+06
Uncharacterized protein	tr E1B919 E1B919_BOVIN;tr G3MWU4 G3MWU4_BOVIN	NaN	24.997	Total vitreous	3.50E+07
Proteasome subunit beta type	tr G5E589 G5E589_BOVIN;sp Q2TBX6 PSB1_BOVIN	NaN	24.999	Total vitreous	3.20E+07
Uncharacterized protein (Fragment)	tr F1MVT9 F1MVT9_BOVIN	NaN	25.014	Total vitreous	5.20E+07
Glia maturation factor beta	sp P60984 GMFB_BOVIN;sp Q56JZ9 GMFG_BOVIN	NaN	25.020	Total vitreous	3.10E+07
Peroxisedoxin-4	sp Q9BG12 PRDX4_BOVIN	NaN			

Protein phosphatase inhibitor 2	tr F1MTZ0 F1MTZ0_BOVIN;sp Q3SZX2 IPP2_BOVIN	NaN	25.029	Total vitreous	6.70E+07
Farnesyl pyrophosphate synthase	sp Q8WMY2 FPPS_BOVIN;tr F1N431 F1N431_BOVIN;tr F1N0Q7 F1N0Q7_BOVIN;tr G3MZT9 G3MZT9_BOVIN	NaN	25.044	Total vitreous	3.20E+07
Glial fibrillary acidic protein	sp Q28115 GFAP_BOVIN	NaN	25.065	Total vitreous	1.70E+07
PGM2 protein (Fragment)	tr A6QQ11 A6QQ11_BOVIN	NaN	25.091	Total vitreous	1.50E+07
UV excision repair protein RAD23 homolog A	sp A3KMV2 RD23A_BOVIN	NaN	25.108	Total vitreous	3.80E+07
Uncharacterized protein	tr E1B8P3 E1B8P3_BOVIN	NaN	25.123	Total vitreous	4.50E+07
Uncharacterized protein (Fragment)	tr F1MY31 F1MY31_BOVIN	NaN	25.129	Total vitreous	1.30E+07
Leukotriene A-4 hydrolase	sp Q3SZH7 LKHA4_BOVIN	NaN	25.152	Total vitreous	1.50E+07
Major prion protein	sp P10279 PRIO_BOVIN	NaN	25.169	Total vitreous	4.20E+07
Neurofilament medium polypeptide	tr F1MDZ2 F1MDZ2_BOVIN;sp O77788 NFM_BOVIN	NaN	25.170	Total vitreous	1.10E+07
G1 to S phase transition 1	tr F6Q087 F6Q087_BOVIN	NaN	25.184	Total vitreous	1.80E+07
Uncharacterized protein	tr F1MRZ5 F1MRZ5_BOVIN;tr F1MRZ6 F1MRZ6_BOVIN	NaN	25.188	Total vitreous	5.90E+06
Branched-chain-amino-acid aminotransferase	tr A4IFQ7 A4IFQ7_BOVIN	NaN	25.204	Total vitreous	2.90E+07

Isopentenyl-diphosphate Delta-isomerase 1	sp Q1LZ95 IDI1_BOVIN;sp Q1LZ95-2 IDI1_BOVIN	NaN	25.216	Total vitreous	4.00E+07
Pikachurin	tr F1N606 F1N606_BOVIN;sp A3KN33 EGFLA_BOVIN	NaN	25.218	Total vitreous	1.20E+07
Argininosuccinate lyase	tr F1MTV7 F1MTV7_BOVIN;sp Q3SZJ0 ARLY_BOVIN	NaN	25.218	Total vitreous	2.10E+07
Lysosomal-associated membrane protein 2	tr Q3SZJ7 Q3SZJ7_BOVIN;tr G3MXJ5 G3MXJ5_BOVIN	NaN	25.219	Total vitreous	3.30E+07
Uncharacterized protein	tr E1BPC9 E1BPC9_BOVIN	NaN	25.224	Total vitreous	1.10E+07
45 kDa calcium-binding protein	sp Q3ZBZ1 CAB45_BOVIN;tr F1MKI5 F1MKI5_BOVIN	NaN	25.230	Total vitreous	3.10E+07
Epiphycan	sp P79119 EPYC_BOVIN	NaN	25.231	Total vitreous	4.40E+07
Reticulon	tr F1N405 F1N405_BOVIN;tr Q7YRW9 Q7YRW9_BOVIN;tr Q1RMR8 Q1RMR8_BOVIN	NaN	25.232	Total vitreous	1.50E+07
Mannose-6-phosphate isomerase	tr F1N327 F1N327_BOVIN;sp Q3SZI0 MPI_BOVIN	NaN	25.232	Total vitreous	2.40E+07
Uncharacterized protein	tr F1MS23 F1MS23_BOVIN	NaN	25.239	Total vitreous	5.30E+07
RNASET2 protein (Fragment)	tr Q0III8 Q0III8_BOVIN	NaN	25.240	Total vitreous	3.10E+07
Enolase-phosphatase E1	sp Q0VD27 ENOPH_BOVIN	NaN	25.264	Total vitreous	4.60E+07
Copper transport protein ATOX1	sp Q3T0E0 ATOX1_BOVIN	NaN	25.284	Total vitreous	1.40E+08

Tyrosine-protein phosphatase non-receptor type substrate 1	sp O46631 SHPS1_BOVIN;tr F1MD75 F1MD75_BOVIN;tr F1MRG6 F1MRG6_BOVIN;tr G3X6M9 G3X6M9_BOVIN	NaN	25.289	Total vitreous	2.40E+07
Uncharacterized protein	tr E1BGX4 E1BGX4_BOVIN	NaN	25.292	Total vitreous	5.20E+07
Insulin-like growth factor II	sp P07456 IGF2_BOVIN	NaN	25.309	Total vitreous	6.20E+07
Uncharacterized protein	tr F1MWN3 F1MWN3_BOVIN	NaN	25.311	Total vitreous	9.20E+06
Kininogen-2	sp P01045 KNG2_BOVIN;CON_P01045-1;sp P01045-2 KNG2_BOVIN	NaN	25.315	Total vitreous	1.80E+07
ECM1 protein	tr A5PJT7 A5PJT7_BOVIN	NaN	25.317	Total vitreous	1.90E+07
Complement component 1, r subcomponent	tr A5D9E9 A5D9E9_BOVIN;tr Q3SYT3 Q3SYT3_BOVIN;tr F1N0N3 F1N0N3_BOVIN	NaN	25.326	Total vitreous	1.60E+07
Uncharacterized protein	tr E1BA06 E1BA06_BOVIN	NaN	25.329	Total vitreous	4.10E+07
Ezrin	sp P31976 EZRI_BOVIN;tr F1MJJ8 F1MJJ8_BOVIN;sp Q32LP2 RADI_BOVIN	NaN	25.380	Total vitreous	1.90E+07
Uncharacterized protein	tr F1N6Y1 F1N6Y1_BOVIN	NaN	25.390	Total vitreous	1.20E+07
Angiotensin-converting enzyme (Fragment)	tr F1MQJ0 F1MQJ0_BOVIN	NaN	25.391	Total vitreous	9.00E+06
Uncharacterized protein	tr F6QJQ2 F6QJQ2_BOVIN	NaN	25.408	Total vitreous	1.40E+07

Peptidyl-prolyl cis-trans isomerase D	sp P26882 PPID_BOVIN	NaN	25.408	Total vitreous	2.60E+07
Uncharacterized protein	tr E1BKN2 E1BKN2_BOVIN	NaN	25.452	Total vitreous	1.10E+07
Uncharacterized protein	tr E1BP81 E1BP81_BOVIN	NaN	25.455	Total vitreous	1.20E+07
Complement factor H (Fragment)	tr F1MC45 F1MC45_BOVIN	NaN	25.455	Total vitreous	1.50E+07
Complement factor D	sp Q3T0A3 CFAD_BOVIN;tr G3N1I8 G3N1I8_BOVIN	NaN	25.463	Total vitreous	4.50E+07
Uncharacterized protein	tr F1MNS8 F1MNS8_BOVIN	NaN	25.470	Total vitreous	9.00E+07
Uncharacterized protein	tr F1MND9 F1MND9_BOVIN	NaN	25.474	Total vitreous	1.30E+07
Galectin-1	sp P11116 LEG1_BOVIN	NaN	25.496	Total vitreous	8.10E+07
F-box only protein 2	sp Q17QK6 FBX2_BOVIN;tr F1M FN6 F1MFN6_BOVIN	NaN	25.505	Total vitreous	5.40E+07
Collagen alpha-1(XI) chain	tr F1N0K0 F1N0K0_BOVIN	NaN	25.510	Total vitreous	7.00E+06
Neurofilament light polypeptide	sp P02548 NFL_BOVIN	NaN	25.566	Total vitreous	1.80E+07
Serpin peptidase inhibitor, clade A (Alpha-1 antitrypsin), member 7	tr Q3SYR0 Q3SYR0_BOVIN;sp Q9TT36 THBG_BOVIN;CON_Q9TT36	NaN	25.570	Total vitreous	3.20E+07
Uncharacterized protein	tr Q3T0Z0 Q3T0Z0_BOVIN;tr G3MX65 G3MX65_BOVIN	NaN	25.572	Total vitreous	8.40E+07

Proliferation-associated 2G4, 38kDa	tr Q3ZBH5 Q3ZBH5_BOVIN	NaN	25.574	Total vitreous	3.10E+07
RAB1A, member RAS oncogene family	tr A1L528 A1L528_BOVIN;tr Q3Z BD1 Q3ZBD1_BOVIN;tr G3MYN 4 G3MYN4_BOVIN;sp Q1RMR4 RAB15_BOVIN	NaN	25.578	Total vitreous	4.70E+07
	tr F1MWQ0 F1MWQ0_BOVIN;sp Q0IIE0 CPLX3_BOVIN	NaN	25.594	Total vitreous	7.60E+07
	sp A2I7N0 SPA34_BOVIN;CON_ _A2I7N0;sp Q3ZEJ6 SPA33_BO VIN	NaN	25.613	Total vitreous	3.50E+07
Serpin A3-4	tr G5E5Y5 G5E5Y5_BOVIN;sp P 61955 SUMO2_BOVIN;sp Q17Q V3 SUMO3_BOVIN;tr G3N303 G 3N303_BOVIN	NaN	25.619	Total vitreous	3.50E+08
Uncharacterized protein	tr E1BIM6 E1BIM6_BOVIN	NaN	25.630	Total vitreous	3.30E+07
Myristoylated alanine-rich C- kinase substrate	tr F1N2N5 F1N2N5_BOVIN;sp P 12624 MARCS_BOVIN;tr E1B91 6 E1B916_BOVIN	NaN	25.648	Total vitreous	5.10E+07
DNA-(apurinic or apyrimidinic site) lyase	sp P23196 APEX1_BOVIN	NaN	25.656	Total vitreous	5.10E+07
Coagulation factor X	sp P00743 FA10_BOVIN	NaN	25.658	Total vitreous	3.40E+07
Phospholysine phosphohistidine inorganic pyrophosphate phosphatase	sp Q0VD18 LHPP_BOVIN	NaN	25.670	Total vitreous	5.10E+07

Tubulin polymerization-promoting protein	sp Q27957 TPPP_BOVIN	NaN	25.671	Total vitreous	6.10E+07
Oligodendrocyte myelin glycoprotein	tr Q0IIH3 Q0IIH3_BOVIN	NaN	25.672	Total vitreous	3.80E+07
Phosphoglycolate phosphatase	sp Q2T9S4 PGP_BOVIN	NaN	25.676	Total vitreous	3.90E+07
Follistatin-related protein 1	sp Q58D84 FSTL1_BOVIN	NaN	25.678	Total vitreous	3.30E+07
ADM	sp O62827 ADML_BOVIN	NaN	25.688	Total vitreous	6.10E+07
Nuclear transport factor 2	sp Q32KP9 NTF2_BOVIN	NaN	25.690	Total vitreous	1.00E+08
Insulin-like growth factor-binding protein 2	sp P13384 IBP2_BOVIN;tr F1N2P8 F1N2P8_BOVIN	NaN	25.691	Total vitreous	4.60E+07
Glutaminyl-peptide cyclotransferase	tr Q0P598 Q0P598_BOVIN;sp Q28120 QPCT_BOVIN	NaN	25.702	Total vitreous	4.30E+07
Uncharacterized protein (Fragment)	tr F1MQJ3 F1MQJ3_BOVIN	NaN	25.706	Total vitreous	3.20E+07
Uncharacterized protein	tr G3MYU9 G3MYU9_BOVIN	NaN	25.729	Total vitreous	3.30E+07
Beta-crystallin A3	tr F1N5Q6 F1N5Q6_BOVIN	NaN	25.742	Total vitreous	6.90E+07
Ubiquitin-conjugating enzyme E2 L3 (Fragment)	tr F1MC72 F1MC72_BOVIN;sp Q3MHP1 UB2L3_BOVIN	NaN	25.764	Total vitreous	9.80E+07
NAD(P)H dehydrogenase, quinone 1	tr Q3ZBH2 Q3ZBH2_BOVIN	NaN	25.774	Total vitreous	5.50E+07
Uncharacterized protein (Fragment)	tr F1MUA1 F1MUA1_BOVIN	NaN	25.774	Total vitreous	6.50E+07

Heterogeneous nuclear ribonucleoprotein A1	sp P09867 ROA1_BOVIN;tr G5E5V7 G5E5V7_BOVIN;tr F1MH29 F1MH29_BOVIN;tr F1MTY3 F1MTY3_BOVIN	NaN	25.815	Total vitreous	4.40E+07
UMP-CMP kinase	sp Q2KIW9 KCY_BOVIN	NaN	25.869	Total vitreous	7.60E+07
Destrin	sp Q5E9D5 DEST_BOVIN	NaN	25.880	Total vitreous	8.00E+07
Uncharacterized protein (Fragment)	tr F1MCF1 F1MCF1_BOVIN;tr F1N7I0 F1N7I0_BOVIN	NaN	25.902	Total vitreous	1.20E+08
Methionine adenosyltransferase 2 subunit beta	sp Q29RI9 MAT2B_BOVIN	NaN	25.906	Total vitreous	4.50E+07
NEDD8 (Fragment)	tr G8JKV8 G8JKV8_BOVIN;sp P61282 NEDD8_BOVIN	NaN	25.913	Total vitreous	2.80E+08
PCSK1N protein	tr A4IFR2 A4IFR2_BOVIN	NaN	25.914	Total vitreous	7.10E+07
Uncharacterized protein	tr E1BI82 E1BI82_BOVIN;CON_Q2HJF0	NaN	25.928	Total vitreous	2.30E+07
Uncharacterized protein	tr F1MCF8 F1MCF8_BOVIN	NaN	25.932	Total vitreous	1.20E+08
C-X-C motif chemokine 16	sp Q29RT9 CXL16_BOVIN	NaN	25.941	Total vitreous	1.10E+08
Uncharacterized protein (Fragment)	tr F1MYW3 F1MYW3_BOVIN	NaN	25.959	Total vitreous	2.10E+07

Uncharacterized protein (Fragment)	tr G3MXG6 G3MXG6_BOVIN;C ON__ENSEMBL:ENSBTAP0000 0033053;tr G3MZH0 G3MZH0_B OVIN;CON__ENSEMBL:ENSBT AP00000011227;tr G3MXD9 G3 MXD9_BOVIN;tr G3N3Q3 G3N3 Q3_BOVIN	NaN	25.967	Total vitreous	1.30E+08
Apolipoprotein A-II	sp P81644 APOA2_BOVIN;CON _P81644	NaN	25.973	Total vitreous	1.80E+08
Cystatin-B	tr F6QEL0 F6QEL0_BOVIN	NaN	25.978	Total vitreous	2.20E+08
Serpin A3-8	sp A6QPQ2 SPA38_BOVIN	NaN	26.000	Total vitreous	3.50E+07
Uncharacterized protein	tr F1MIQ2 F1MIQ2_BOVIN	NaN	26.033	Total vitreous	2.10E+07
Uncharacterized protein (Fragment)	tr F1MF04 F1MF04_BOVIN	NaN	26.035	Total vitreous	2.80E+07
Tubulin-specific chaperone A	sp P48427 TBCA_BOVIN	NaN	26.060	Total vitreous	1.60E+08
Stathmin	sp Q3T0C7 STMN1_BOVIN;tr F1 N1C2 F1N1C2_BOVIN;tr Q3MHJ 8 Q3MHJ8_BOVIN	NaN	26.062	Total vitreous	1.10E+08
Neurosecretory protein VGF	tr F2Z4E0 F2Z4E0_BOVIN;sp P8 6435 VGF_BOVIN	NaN	26.085	Total vitreous	3.40E+07
Uncharacterized protein	tr E1BLA8 E1BLA8_BOVIN	NaN	26.102	Total vitreous	4.00E+07
Peptidyl-prolyl cis-trans isomerase FKBP1A	sp P18203 FKB1A_BOVIN;tr Q2 NKS8 Q2NKS8_BOVIN	NaN	26.137	Total vitreous	3.30E+08

Uncharacterized protein (Fragment)	tr F1MPD1 F1MPD1_BOVIN	NaN	26.161	Total vitreous	1.80E+07
Ephrin-A1	sp Q3ZC64 EFNA1_BOVIN	NaN	26.169	Total vitreous	1.10E+08
14 kDa phosphohistidine phosphatase	sp Q32PA4 PHP14_BOVIN	NaN	26.169	Total vitreous	1.10E+08
Profilin	tr E1BHJ0 E1BHJ0_BOVIN;sp P 02584 PROF1_BOVIN;CON_P 02584	NaN	26.169	Total vitreous	1.10E+08
Uncharacterized protein (Fragment)	tr F6QDM0 F6QDM0_BOVIN	NaN	26.197	Total vitreous	9.40E+07
Heterogeneous nuclear ribonucleoprotein D	tr A5D9H5 A5D9H5_BOVIN;tr A6 H6Y0 A6H6Y0_BOVIN;tr F1N2T 0 F1N2T0_BOVIN	NaN	26.209	Total vitreous	7.50E+07
Isoform ACY1 of Acylphosphatase-1	sp P41500- 2 ACYP1_BOVIN;sp P41500 AC YP1_BOVIN	NaN	26.220	Total vitreous	1.50E+08
Mast/stem cell growth factor receptor Kit	sp P43481 KIT_BOVIN	NaN	26.233	Total vitreous	2.00E+07
Uncharacterized protein (Fragment)	tr F1MFJ3 F1MFJ3_BOVIN;tr G3 MXS7 G3MXS7_BOVIN	NaN	26.239	Total vitreous	1.80E+07
Myotrophin	sp Q3T0F7 MTPN_BOVIN	NaN	26.249	Total vitreous	2.00E+08
Uncharacterized protein (Fragment)	tr E1BE12 E1BE12_BOVIN	NaN	26.260	Total vitreous	2.40E+07
Uncharacterized protein	tr F1N739 F1N739_BOVIN;tr F1 N279 F1N279_BOVIN	NaN	26.290	Total vitreous	1.50E+07
MSLN protein	tr A6QP39 A6QP39_BOVIN	NaN	26.293	Total vitreous	5.10E+07

Uncharacterized protein	tr F1MDC2 F1MDC2_BOVIN	NaN	26.318	Total	2.80E+07
Aldehyde dehydrogenase	tr F1N015 F1N015_BOVIN;sp P30907 AL3A1_BOVIN	NaN	26.320	Total	5.60E+07
Similar to hemopexin	CON_Q3SZV7	NaN	26.325	Total	5.90E+07
Uncharacterized protein (Fragment)	tr F1MNL4 F1MNL4_BOVIN	NaN	26.329	Total	1.90E+07
Interphotoreceptor matrix proteoglycan 1	sp Q9GMS5 IMPG1_BOVIN;tr G3N2H2 G3N2H2_BOVIN;tr F1MY68 F1MY68_BOVIN	NaN	26.419	Total	3.10E+07
SH3 domain-binding glutamic acid-rich-like protein 3	sp Q3ZCL8 SH3L3_BOVIN;tr G3X6S5 G3X6S5_BOVIN	NaN	26.432	Total	3.10E+08
Uncharacterized protein	tr F1MEH3 F1MEH3_BOVIN	NaN	26.438	Total	6.50E+07
Uncharacterized protein	tr G3MYP5 G3MYP5_BOVIN;tr F1N3M8 F1N3M8_BOVIN	NaN	26.441	Total	9.50E+06
LMAN2 protein	tr A6QP36 A6QP36_BOVIN	NaN	26.464	Total	6.30E+07
Uncharacterized protein	tr F1MGK5 F1MGK5_BOVIN	NaN	26.486	Total	1.60E+07
Retinol-binding protein 1	sp P02694 RET1_BOVIN	NaN	26.519	Total	1.40E+08
Beta-crystallin B1	sp P07318 CRBB1_BOVIN;tr E1BFK5 E1BFK5_BOVIN	NaN	26.545	Total	8.30E+07
CALR protein	tr A5D7J6 A5D7J6_BOVIN;sp P52193 CALR_BOVIN	NaN	26.599	Total	6.00E+07

Pantetheinase	sp Q58CQ9 VNN1_BOVIN	NaN	26.609	Total	6.90E+07
Ester hydrolase C11orf54 homolog	sp Q2HJH3 CK054_BOVIN	NaN	26.615	Total	9.90E+07
Uncharacterized protein	tr E1BBM1 E1BBM1_BOVIN	NaN	26.636	Total	6.70E+07
Cadherin-6	sp Q3SWX5 CADH6_BOVIN	NaN	26.638	Total	4.50E+07
Fatty acid-binding protein, heart	sp P10790 FABPH_BOVIN;tr F1MHQ4 F1MHQ4_BOVIN;sp P48035 FABP4_BOVIN;sp P02690 MYP2_BOVIN	NaN	26.657	Total	1.50E+08
Uncharacterized protein (Fragment)	tr G3N3P6 G3N3P6_BOVIN	NaN	26.674	Total	3.20E+08
CSTB protein	tr A6QPZ0 A6QPZ0_BOVIN;sp P35478 CYTX_BOVIN	NaN	26.695	Total	2.40E+08
Uncharacterized protein (Fragment)	tr H9GW42 H9GW42_BOVIN	NaN	26.697	Total	1.60E+08
Alpha-N-acetylgalactosaminidase	tr Q1RMM9 Q1RMM9_BOVIN;sp Q58DH9 NAGAB_BOVIN	NaN	26.701	Total	6.40E+07
Arylsulfatase A	sp Q08DD1 ARSA_BOVIN	NaN	26.726	Total	1.40E+08
Malic enzyme	tr F1N3V0 F1N3V0_BOVIN	NaN	26.785	Total	4.90E+07
Uncharacterized protein	tr G8JKW3 G8JKW3_BOVIN;sp P62326 TYB4_BOVIN	NaN	26.866	Total	8.20E+08
Uncharacterized protein	tr F1N1S2 F1N1S2_BOVIN	NaN	26.883	Total	1.70E+07

Zinc-alpha-2-glycoprotein	sp Q3ZCH5 ZA2G_BOVIN	NaN	26.958	Total vitreous	1.50E+08
Dihydropteridine reductase	sp Q3T0Z7 DHPR_BOVIN	NaN	26.975	Total vitreous	1.30E+08
Isocitrate dehydrogenase [NADP] cytoplasmic	sp Q9XSG3 IDHC_BOVIN;sp Q04467 IDHP_BOVIN	NaN	26.978	Total vitreous	6.60E+07
CD44 antigen	tr F1MHC3 F1MHC3_BOVIN;sp Q29423 CD44_BOVIN;tr Q0VDD03 Q0VDD03_BOVIN;tr F1MQT9 F1MQT9_BOVIN	NaN	26.984	Total vitreous	9.90E+07
Gastrin-releasing peptide	sp Q863C3 GRP_BOVIN	NaN	27.001	Total vitreous	2.30E+08
Uncharacterized protein	tr E1BF00 E1BF00_BOVIN	NaN	27.036	Total vitreous	2.70E+07
Carbonic anhydrase 2 (Fragment)	tr F1N0H3 F1N0H3_BOVIN	NaN	27.060	Total vitreous	1.30E+08
Uncharacterized protein	tr F1MET4 F1MET4_BOVIN	NaN	27.065	Total vitreous	4.70E+07
SPARC	sp P13213 SPRC_BOVIN	NaN	27.081	Total vitreous	1.50E+08
Transaldolase	tr G5E5C8 G5E5C8_BOVIN;sp Q2TBL6 TALDO_BOVIN;tr Q58DR3 Q58DR3_BOVIN	NaN	27.122	Total vitreous	1.10E+08
Uncharacterized protein	tr F1MSA4 F1MSA4_BOVIN	NaN	27.173	Total vitreous	4.30E+07
Translationally-controlled tumor protein	sp Q5E984 TCTP_BOVIN	NaN	27.189	Total vitreous	2.90E+08

Brain ribonuclease	sp P39873 RNBR_BOVIN;sp P61823 RNAS1_BOVIN;sp P00669 RNS_BOVIN	NaN	27.196	Total vitreous	3.50E+08
Uncharacterized protein	tr F1MEW6 F1MEW6_BOVIN	NaN	27.211	Total vitreous	1.40E+08
Isoform Tau-F of Microtubule-associated protein tau	sp P29172-6 TAU_BOVIN;sp P29172-11 TAU_BOVIN;sp P29172-17 TAU_BOVIN;sp P29172-14 TAU_BOVIN;sp P29172-3 TAU_BOVIN;sp P29172-20 TAU_BOVIN	NaN	27.221	Total vitreous	1.20E+08
	tr Q58DP6 Q58DP6_BOVIN;sp P15467 RNAS4_BOVIN	NaN	27.227	Total vitreous	3.00E+08
	tr F1MVK1 F1MVK1_BOVIN;tr F1MP09 F1MP09_BOVIN	NaN	27.246	Total vitreous	2.70E+07
	sp P55859 PNPH_BOVIN;tr G3X8C8 G3X8C8_BOVIN	NaN	27.287	Total vitreous	1.40E+08
Secretogranin V (7B2 protein)	tr Q2HJG0 Q2HJG0_BOVIN	NaN	27.306	Total vitreous	2.50E+08
Serpin B6	tr F1N2A2 F1N2A2_BOVIN;sp O02739 SPB6_BOVIN;tr F1N0T3 F1N0T3_BOVIN	NaN	27.312	Total vitreous	1.00E+08
CDKL1 protein	tr A6QLF0 A6QLF0_BOVIN	NaN	27.312	Total vitreous	1.10E+08
Protein S100-A4	sp P35466 S10A4_BOVIN	NaN	27.327	Total vitreous	3.80E+08
Thioredoxin (Fragment)	tr G8JKZ8 G8JKZ8_BOVIN;sp O97680 THIO_BOVIN	NaN	27.383	Total vitreous	3.40E+08

Primary amine oxidase, liver isozyme	sp Q29437 AOCX_BOVIN;tr E1BC10 E1BC10_BOVIN;sp Q9TTK6 AOC3_BOVIN;tr E1BD43 E1BD43_BOVIN;sp Q9TTK6-D43_BOVIN;sp Q9TTK6-2 AOC3_BOVIN;tr E1BJN3 E1BJN3_BOVIN;sp O46406 AOCY_BN3_BOVIN;sp O46406 AOCY_BOVIN;tr G3MX04 G3MX04_BOVIN;tr E1BC09 E1BC09_BOVIN;N;tr E1BC09 E1BC09_BOVIN	NaN	27.383	Total vitreous	7.40E+07
Creatine kinase M-type	sp Q9XSC6 KCRM_BOVIN	NaN	27.408	Total vitreous	1.50E+08
Cell adhesion molecule 1	tr Q2TBL2 Q2TBL2_BOVIN	NaN	27.419	Total vitreous	1.10E+08
SPOCK2 protein	tr A7MB04 A7MB04_BOVIN	NaN	27.445	Total vitreous	1.10E+08
Purkinje cell protein 4	sp Q148C4 PCP4_BOVIN	NaN	27.504	Total vitreous	6.40E+08
Heat shock 70 kDa protein 1A	sp Q27975 HS71A_BOVIN;sp Q27965 HS71B_BOVIN	NaN	27.529	Total vitreous	7.70E+07
Isoform 2 of Hydroxyacylglutathione hydrolase, mitochondrial	sp Q3B7M2-2 GLO2_BOVIN;sp Q3B7M2 GLO2_BOVIN	NaN	27.535	Total vitreous	1.90E+08
Neurotrimin	sp Q58DA5 NTRI_BOVIN	NaN	27.541	Total vitreous	1.50E+08
Uncharacterized protein	tr E1BPW7 E1BPW7_BOVIN	NaN	27.620	Total vitreous	1.80E+08
Beta-synuclein	tr F1MS41 F1MS41_BOVIN;sp P33567 SYUB_BOVIN	NaN	27.637	Total vitreous	4.70E+08
Uncharacterized protein	tr G5E5V1 G5E5V1_BOVIN	NaN	27.673	Total vitreous	7.20E+08

Seizure 6-like protein 2	sp Q29RN8 SE6L2_BOVIN	NaN	27.699	Total	1.10E+08
Uncharacterized protein (Fragment)	tr F1MZ78 F1MZ78_BOVIN	NaN	27.701	Total	6.30E+07
Protein HP-25 homolog 2	sp Q2KIU3 HP252_BOVIN;CON_Q2KIU3	NaN	27.760	Total	4.00E+08
Secretogranin-1	sp P23389 SCG1_BOVIN	NaN	27.817	Total	9.40E+07
Alcohol dehydrogenase [NADP(+)]	sp Q3ZCJ2 AK1A1_BOVIN	NaN	27.873	Total	1.40E+08
Acidic leucine-rich nuclear phosphoprotein 32 family member A	sp P51122 AN32A_BOVIN	NaN	27.880	Total	3.70E+08
Uncharacterized protein (Fragment)	tr G3X7F3 G3X7F3_BOVIN	NaN	27.882	Total	1.00E+08
Protein HP-25 homolog 1	sp Q2KIX7 HP251_BOVIN	NaN	27.902	Total	4.80E+08
Tubulin alpha-1D chain	sp Q2HJ86 TBA1D_BOVIN	NaN	28.087	Total	2.30E+08
Aldo-keto reductase family 1, member B1	tr Q5E962 Q5E962_BOVIN;sp P16116 ALDR_BOVIN;tr E1BNW1 E1BNW1_BOVIN	NaN	28.196	Total	2.10E+08
Carbonic anhydrase 3	sp Q3SZX4 CAH3_BOVIN	NaN	28.197	Total	2.60E+08
Uncharacterized protein	tr F1MPP2 F1MPP2_BOVIN	NaN	28.290	Total	3.00E+08
Uncharacterized protein	tr E1BAU6 E1BAU6_BOVIN	NaN	28.323	Total	1.70E+08

Insulin-like growth factor-binding protein 5	sp Q05717 IBP5_BOVIN	NaN	28.330	Total vitreous	3.80E+08
Uncharacterized protein	tr E1BNR9 E1BNR9_BOVIN	NaN	28.337	Total vitreous	1.40E+08
Tetranectin	sp Q2KIS7 TETN_BOVIN;CON_Q2KIS7	NaN	28.373	Total vitreous	5.20E+08
Epididymal secretory protein E1	sp P79345 NPC2_BOVIN	NaN	28.512	Total vitreous	5.70E+08
Beta-crystallin B3	sp P19141 CRBB3_BOVIN	NaN	28.584	Total vitreous	4.50E+08
Acyl-CoA-binding protein	sp P07107 ACBP_BOVIN	NaN	28.692	Total vitreous	1.20E+09
Zeta-crystallin	sp O97764 QOR_BOVIN	NaN	28.709	Total vitreous	3.00E+08
Nucleobindin-1	sp Q0P569 NUCB1_BOVIN	NaN	28.771	Total vitreous	2.20E+08
GLO1 protein	tr A4FUZ1 A4FUZ1_BOVIN	NaN	28.857	Total vitreous	6.00E+08
>P00761 SWISS-PROT:P00761	CON_P00761	NaN	29.035	Total vitreous	1.20E+09
>P31096 SWISS-PROT:P31096 Osteopontin - Bos taurus (Bovine).	CON_P31096;tr F1MI46 F1MI46_BOVIN;sp P31096 OSTP_BOVIN;sp P31098 OSTK_BOVIN;tr Q58DM6 Q58DM6_BOVIN	NaN	29.081	Total vitreous	7.30E+08
Myoglobin	sp P02192 MYG_BOVIN	NaN	29.235	Total vitreous	9.50E+08
Uncharacterized protein	tr F6QLF1 F6QLF1_BOVIN;tr F1N2J5 F1N2J5_BOVIN	NaN	29.258	Total vitreous	3.90E+08

Alpha-1B-glycoprotein	sp Q2KJF1 A1BG_BOVIN;CON_ _Q2KJF1	NaN	29.537	Total vitreous	5.90E+08
Secretogranin-2	sp P20616 SCG2_BOVIN	NaN	29.797	Total vitreous	3.60E+08
Aldehyde dehydrogenase 18 family, member A1	tr Q2KJH7 Q2KJH7_BOVIN	NaN	29.803	Total vitreous	2.60E+08
Secretogranin-3	sp A6QLI2 SCG3_BOVIN	NaN	30.079	Total vitreous	6.50E+08
Chromogranin-A	sp P05059 CMGA_BOVIN	NaN	30.124	Total vitreous	5.30E+08
14-3-3 protein sigma	sp Q0VC36 1433S_BOVIN	NaN	30.380	Total vitreous	1.10E+09

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WHAT IS CLAIMED IS:

1. A composition comprising:

one or more aqueous humor and/or vitreous humor extracellular vesicle bodies,
wherein said extracellular vesicle bodies are modified to contain one or more exogenous agents.

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2. The composition of claim 1, wherein the one or more exogenous agents is
selected from the group consisting of a nucleic acid molecule, a protein or polypeptide, a small
molecule, a hormone, and any combination thereof.

10 3. The composition of claim 2, wherein the exogenous agent comprises a
nucleic acid molecule selected from the group consisting of a ribonucleic acid, small RNA
molecule, complementary RNA, a non-coding RNA molecule, siRNA, a pi-RNA molecule, a
micro-RNA molecule, a sno-RNA molecule, long non-coding RNA molecule, messenger RNA
molecule, ribosomal RNA molecule, an antisense nucleic acid molecule, Locked Nucleic Acid
15 (LNA), antagomir, CRISPR/Cas gene editing RNA, trans-activating crRNA (tracrRNA), short
synthetic RNA composed of a "scaffold" sequence (gRNA), Small Cajal body-specific RNAs
(scaRNA), natural cis-antisense siRNAs (cis-nat-siRNAs), trans-acting siRNA (tasiRNA), repeat
associated small interfering RNA (rasiRNA), 7SK, transfer-messenger RNA (tmRNA), transfer
RNA (tRNA), 7SL RNA, signal recognition particle RNA (SRP), and any combination thereof.

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4. The composition of claim 2, wherein the exogenous agent comprises a
small deoxy-ribonucleic acid (DNA) molecule, a cDNA molecule, an oligonucleotide, a locked
Nucleic Acid (LNA), a deoxyribonucleic acid aptamer, a deoxyribonucleic acidzyme, and any
combination thereof.

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5. The composition of any one of claims 1-4, wherein the exogenous agent is
carried in a viral vector, bacterial vector, plasmid vector, or any combination thereof.

6. The composition of claim 1, wherein the exogenous agent comprises a
30 protein or polypeptide.

7. The composition of claim 1, wherein the exogenous agent comprises a
small molecule.

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8. The composition of claim 1, wherein the one or more extracellular vesicle bodies are isolated from ocular fluids containing the aqueous humor and/or the vitreous humor of a mammalian subject.

5 9. The composition of the claim 8, wherein the mammalian subject is a human subject or a bovine subject.

10 10. The composition of claim 1, wherein the one or more extracellular vesicle bodies are further modified to display a eukaryotic cell-specific targeting molecule on the vesicular body outer surface.

11. The composition of claim 1, wherein the exogenous agent comprises a therapeutic agent, said composition further comprising:
a pharmaceutically acceptable carrier.

12. The composition of claim 1, wherein said composition is formulated in a slow or sustained release material.

13. A method of delivering a therapeutic agent to select cells or tissue of a subject, said method comprising:
providing the composition of any one of claims 1-12, wherein said exogenous agent comprises a therapeutic agent and
administering said composition to the subject under conditions effective to deliver the aqueous humor and/or vitreous humor extracellular vesicle bodies modified to contain a therapeutic agent to the select cells or tissue of the subject.

14. The method of claim 13 further comprising:
selecting a subject having an ocular disease, wherein said administering is carried out to deliver the therapeutic agent to the subject's ocular cells or tissue as a treatment for said ocular disease.

15. The method of claim 14, wherein said administration is selected from topical administration, systemic administration, periocular administration, or intraocular administration.

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16. The method of claim 15, wherein said intraocular administration is carried out via intracameral administration, intravitreal administration, or subretinal administration.

17. The method of claim 15, wherein said periocular administration is carried out via sub-conjunctival injection, sub-Tenon's injection, direct periocular injection, or depot periocular injection.

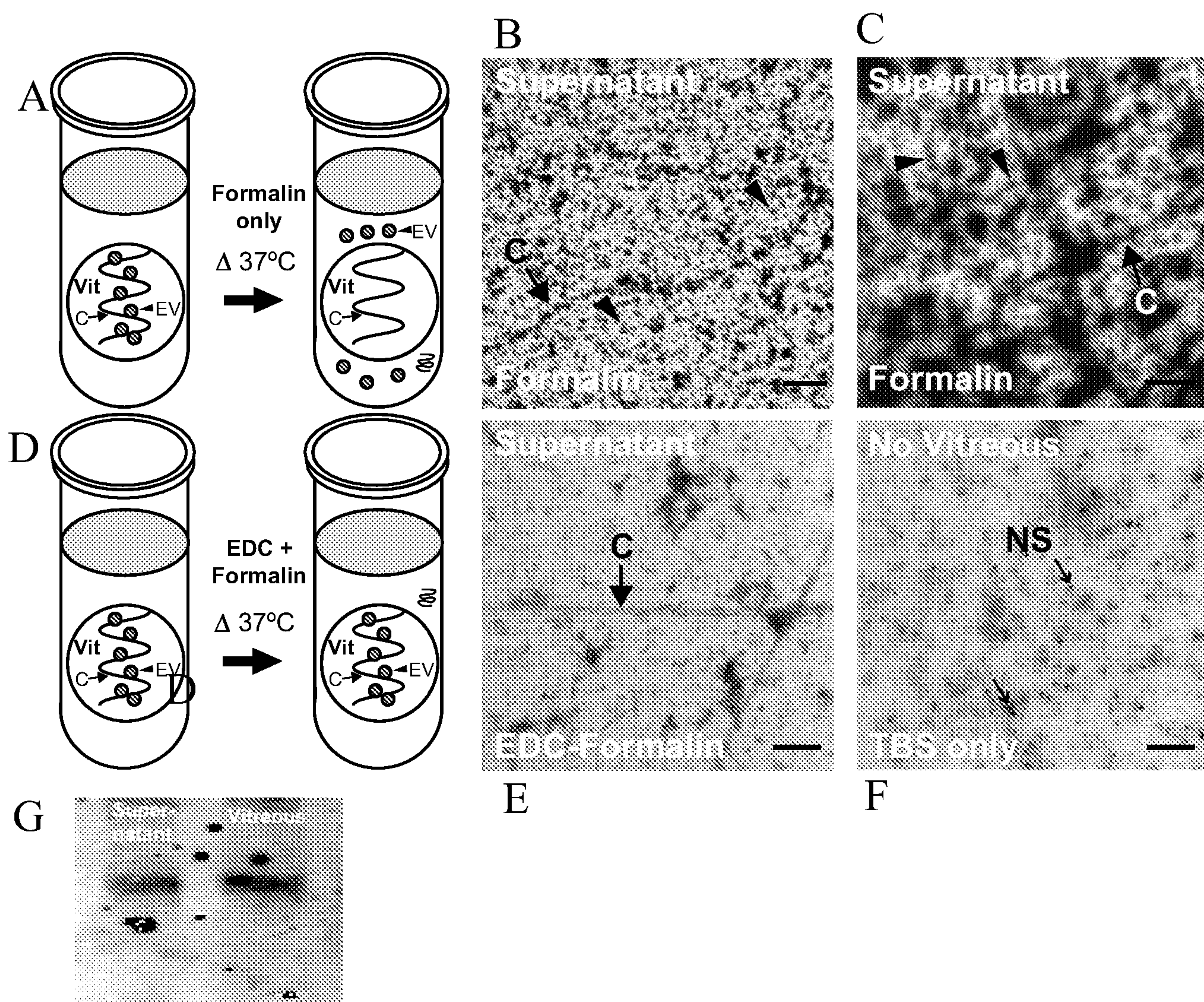
18. The method of claim 15, wherein said systemic administration is carried out via intravenous administration, oral administration, intraarterial administration, inhalation, intranasal administration, intra-peritoneal administration, intra-abdominal administration, subcutaneous administration, intra-articular administration, intrathecal administration, transdural administration, transdermal administration, submucosal administration, sublingual administration, enteral administration, parenteral administration, percutaneous administration, periarticular administration, or intraventricular administration.

19. A method of making the composition of claim 1:
providing a mammalian ocular fluid sample comprising vitreous and/or aqueous humor fluids;
isolating extracellular vesicle bodies from said ocular fluid sample; and
inserting the one or more exogenous agents into the isolated extracellular vesicle bodies.

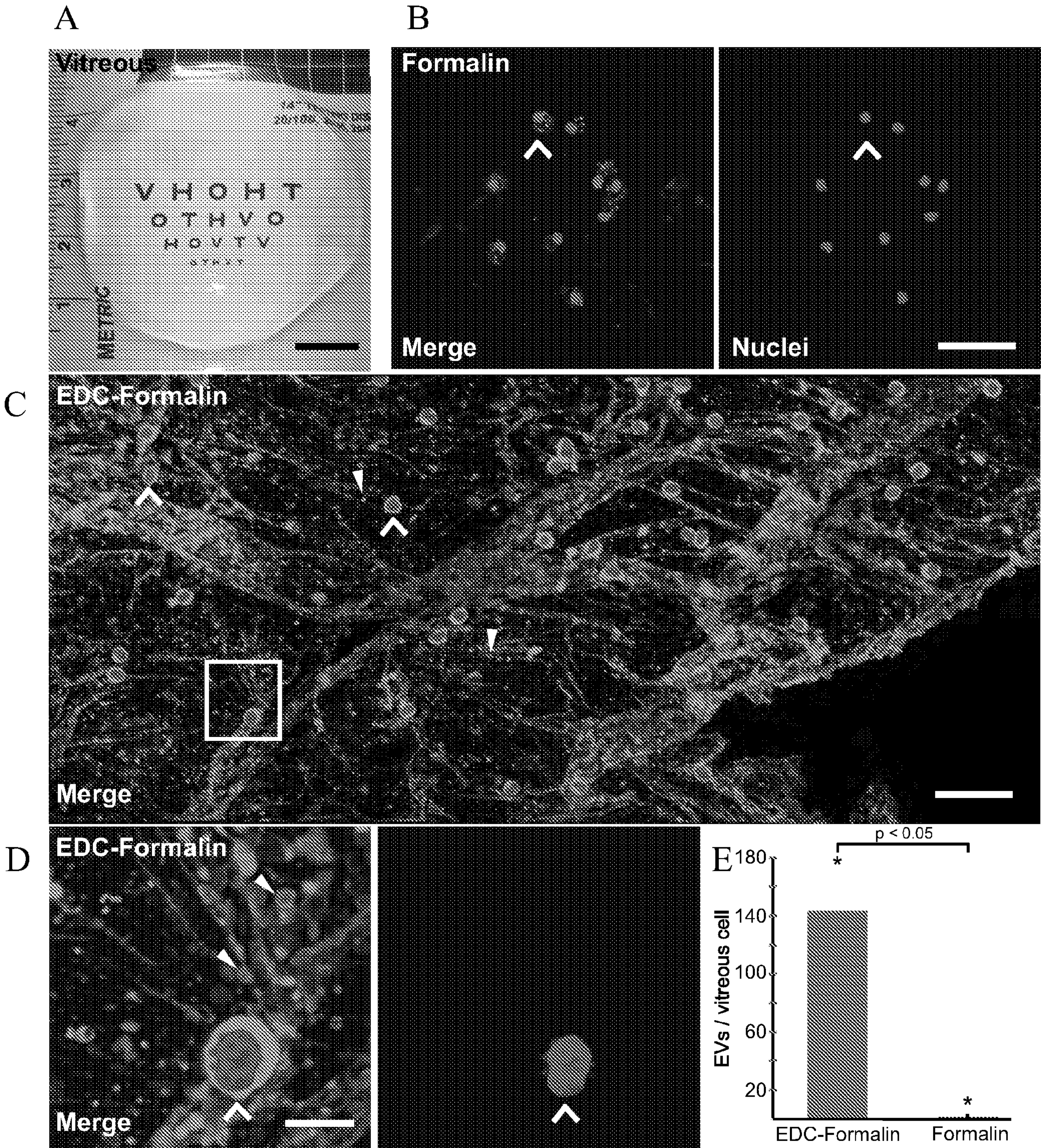
20. The method of claim 19, wherein said inserting is carried out using electroporation, transfection, viral-vector delivery, or any combination thereof.

21. The method of claim 19 further comprising:
removing the endogenous contents of the isolated extracellular vesicle bodies prior to said inserting.

22. The method of claim 21, wherein said removing is carried out using ultraviolet radiation.



FIGs. 1A-1G



FIGs. 2A-2E

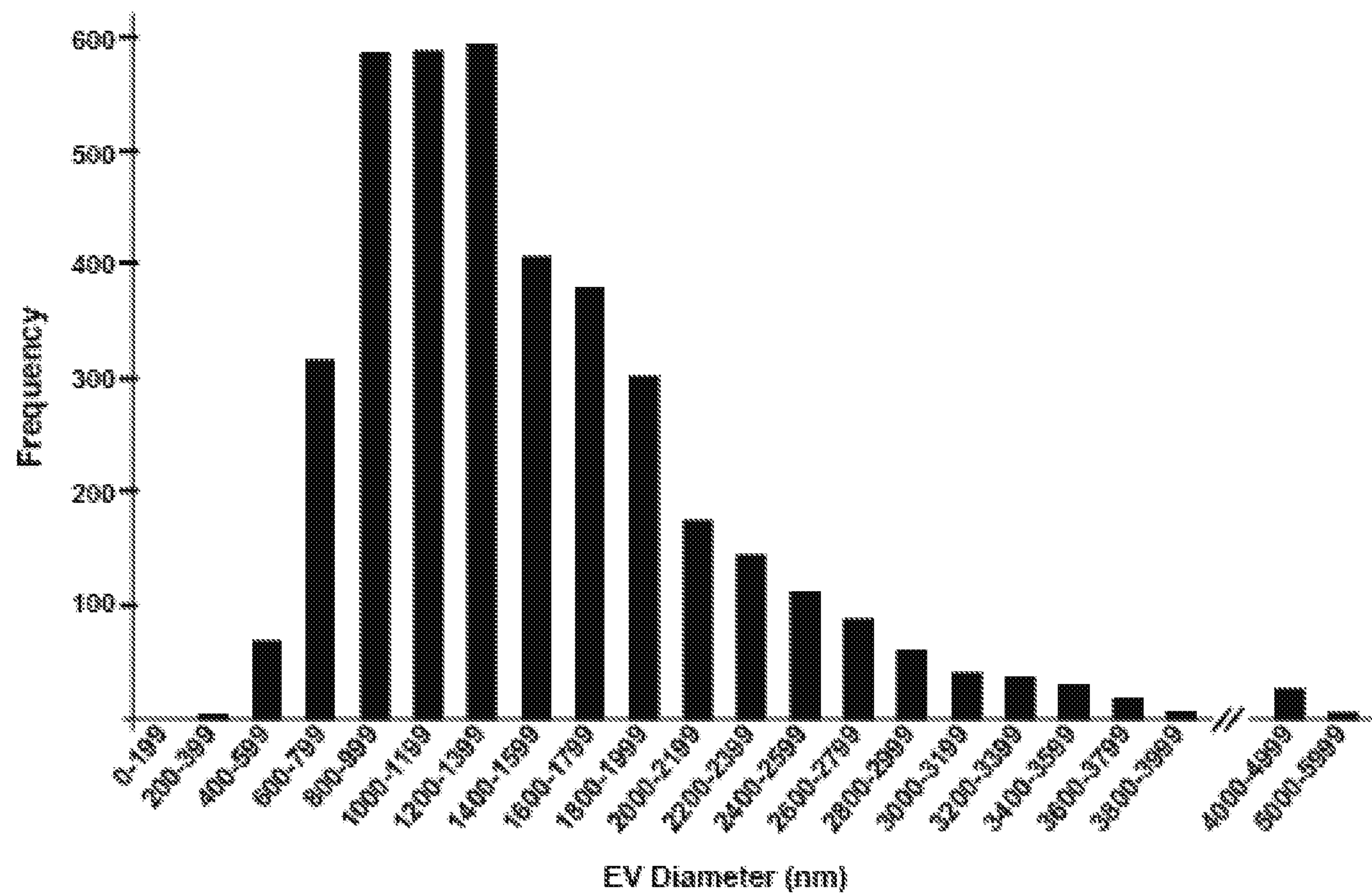
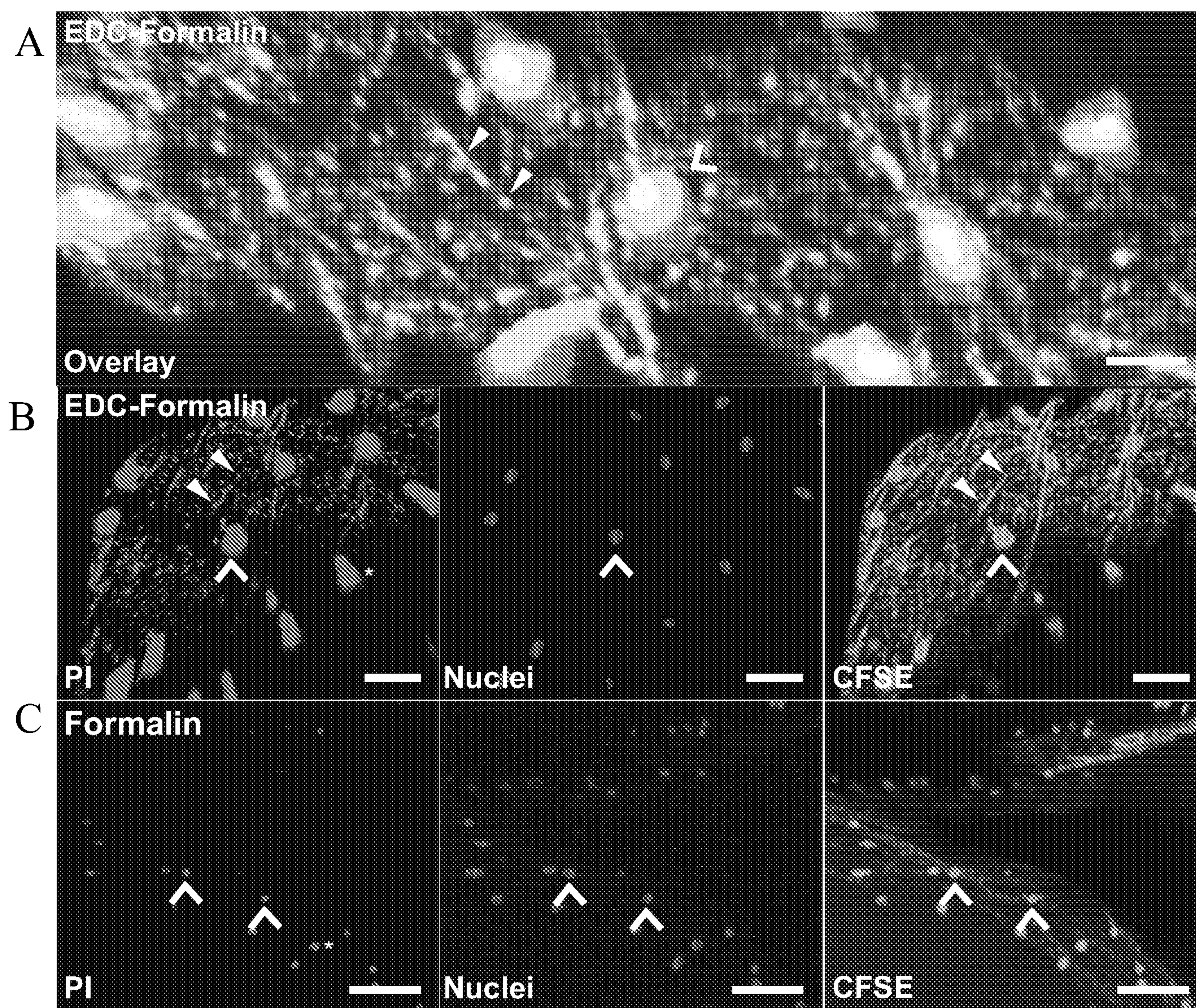
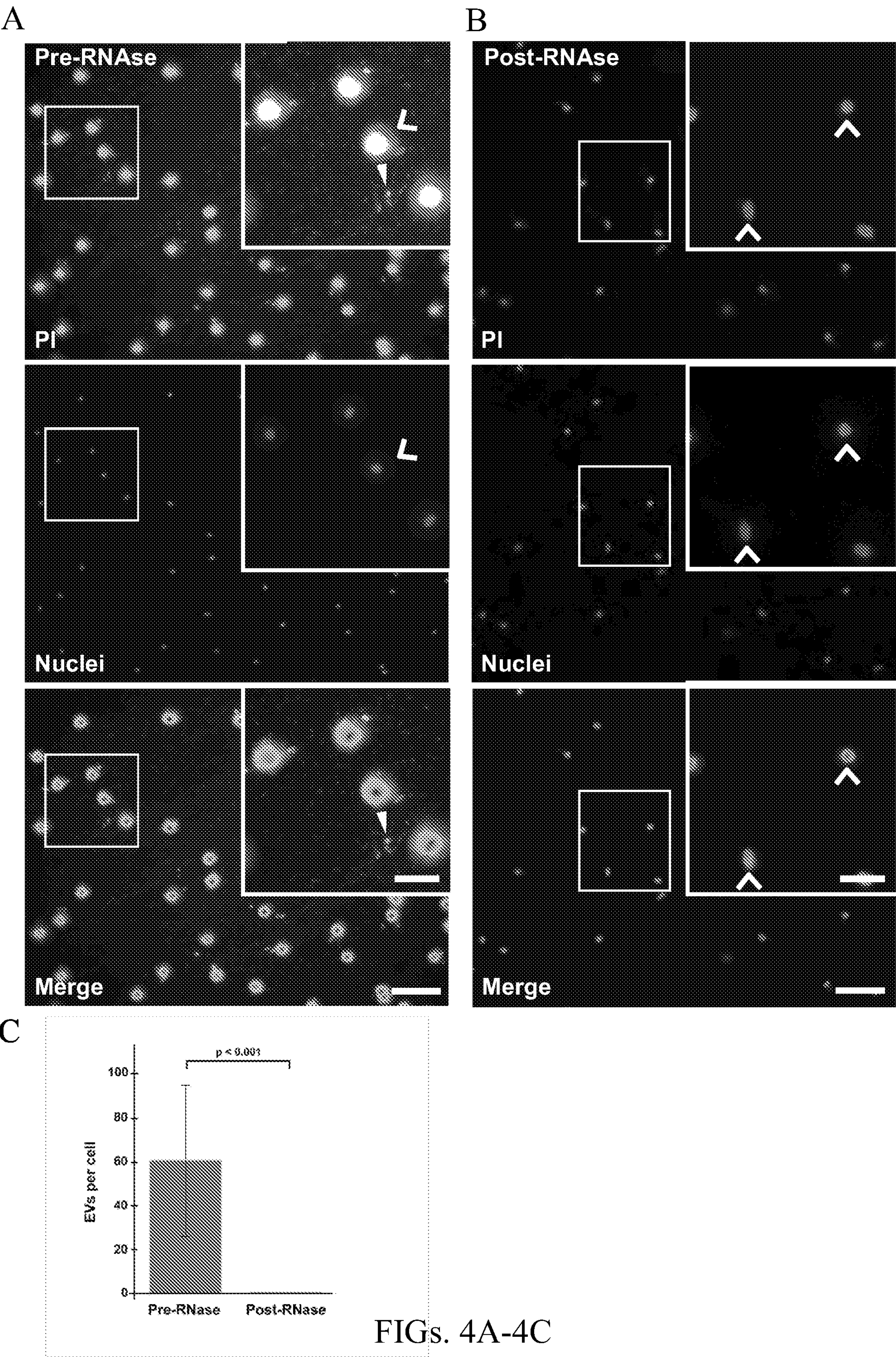


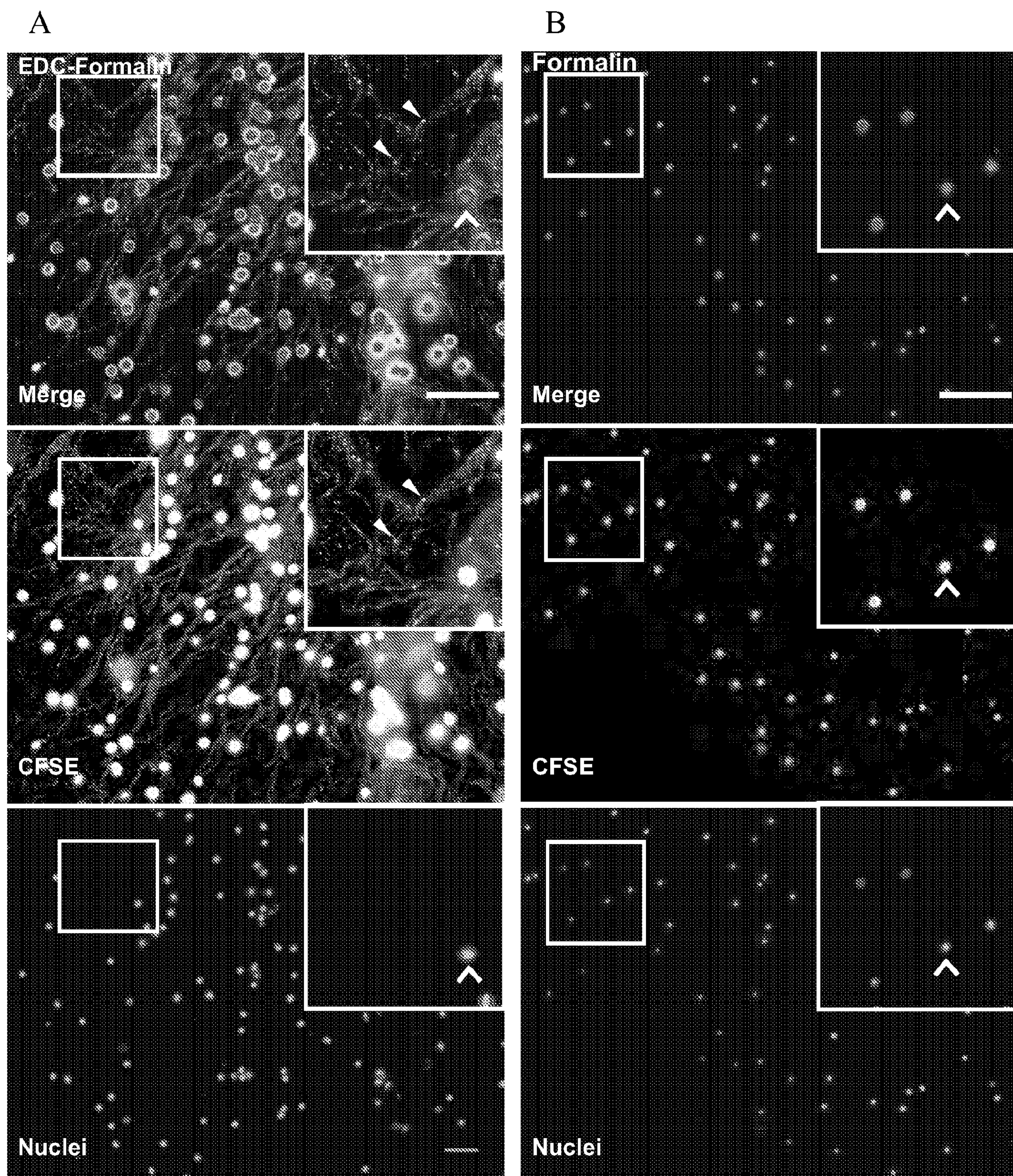
FIG. 2F



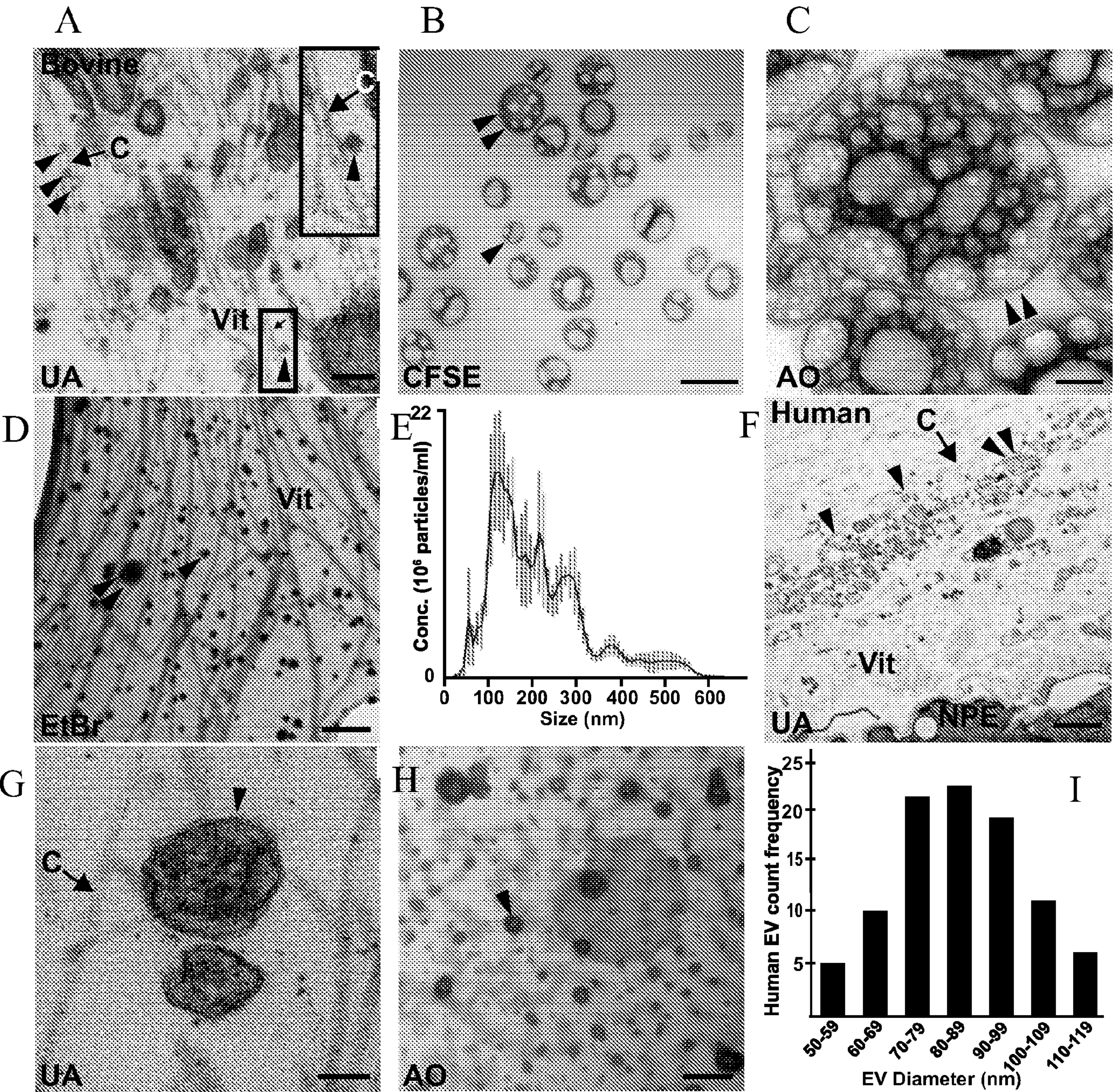
FIGs. 3A-3C



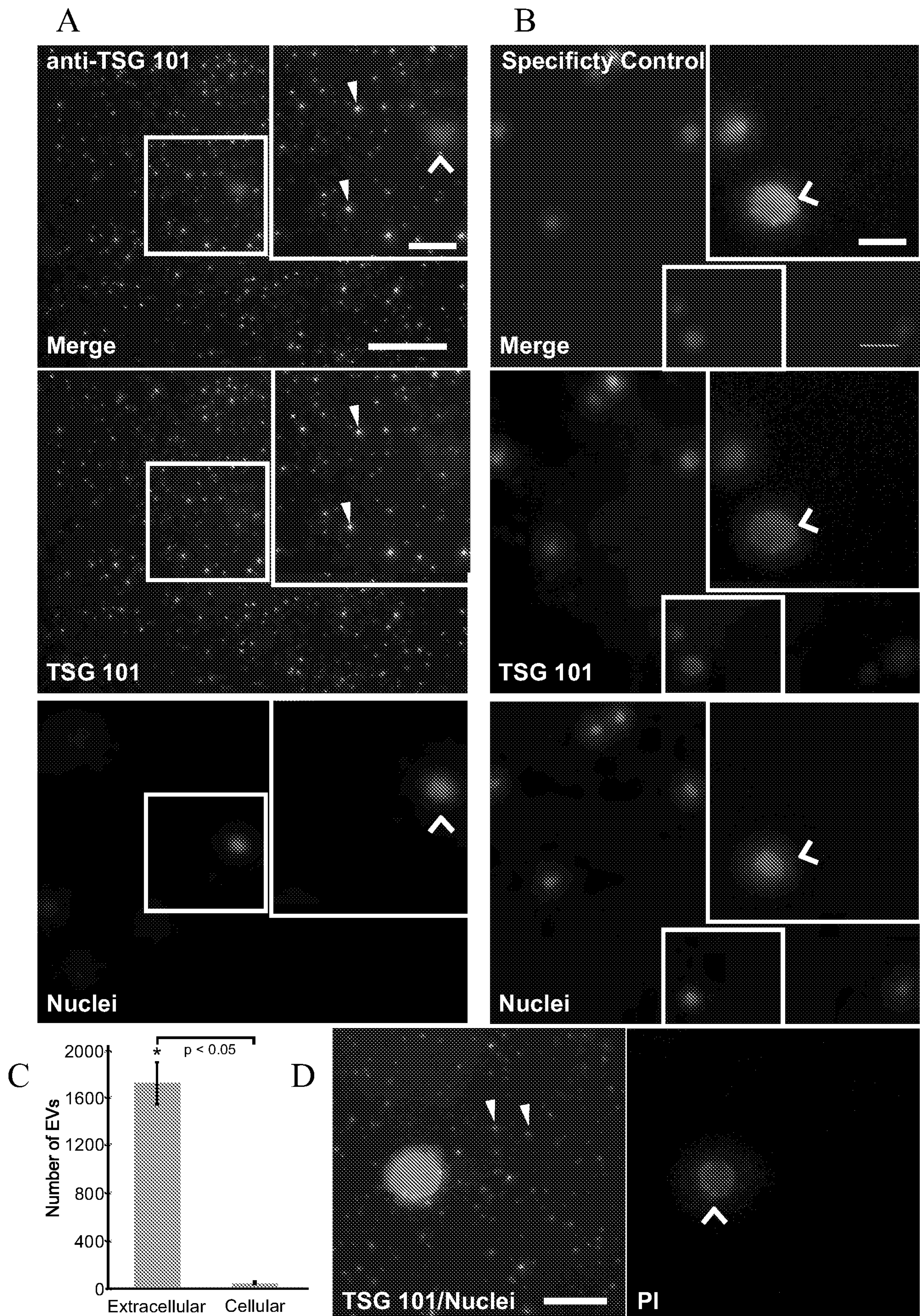
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FIGs. 5A-5B

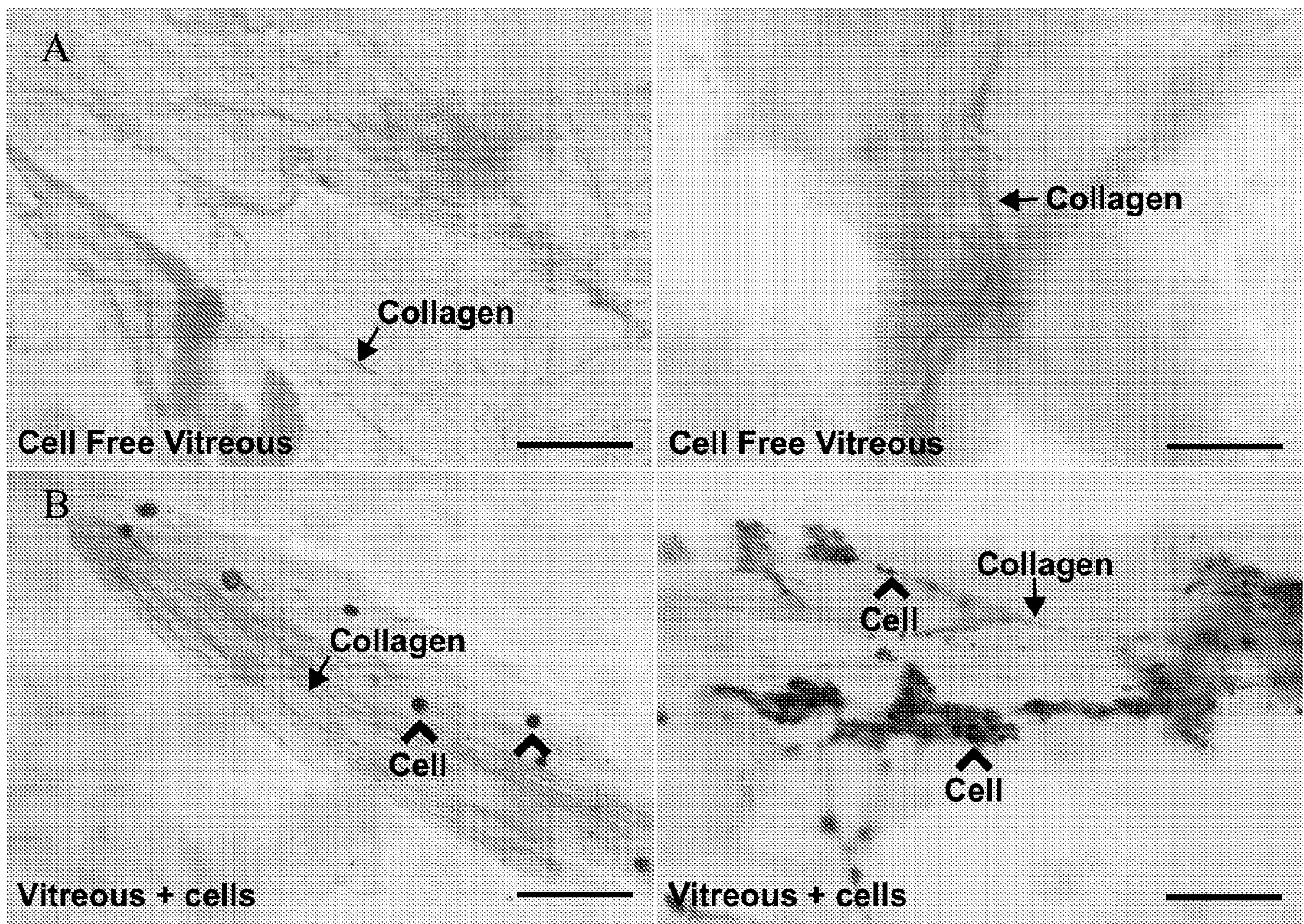


FIGs. 6A-6I

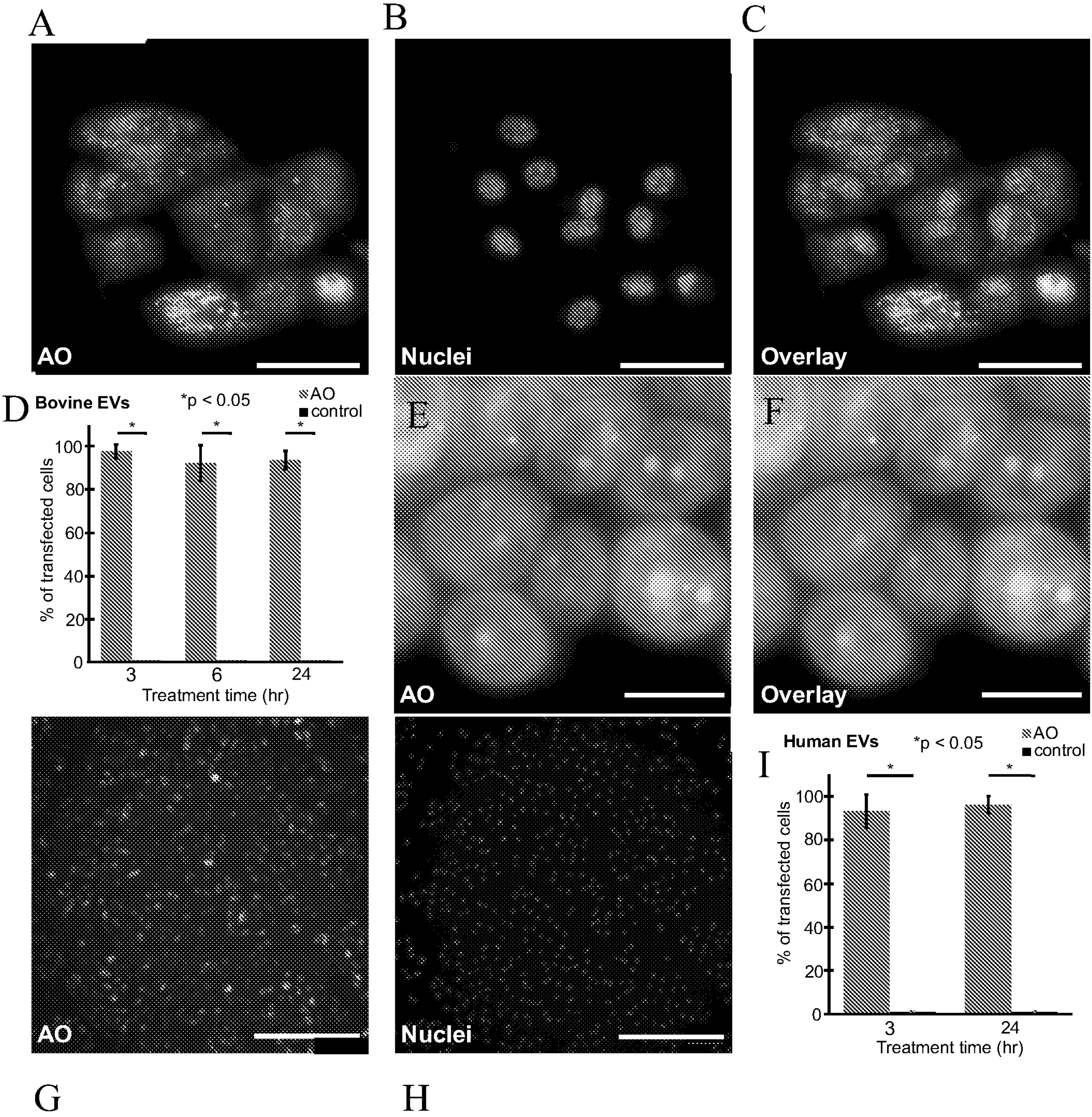


FIGs. 7A-7D

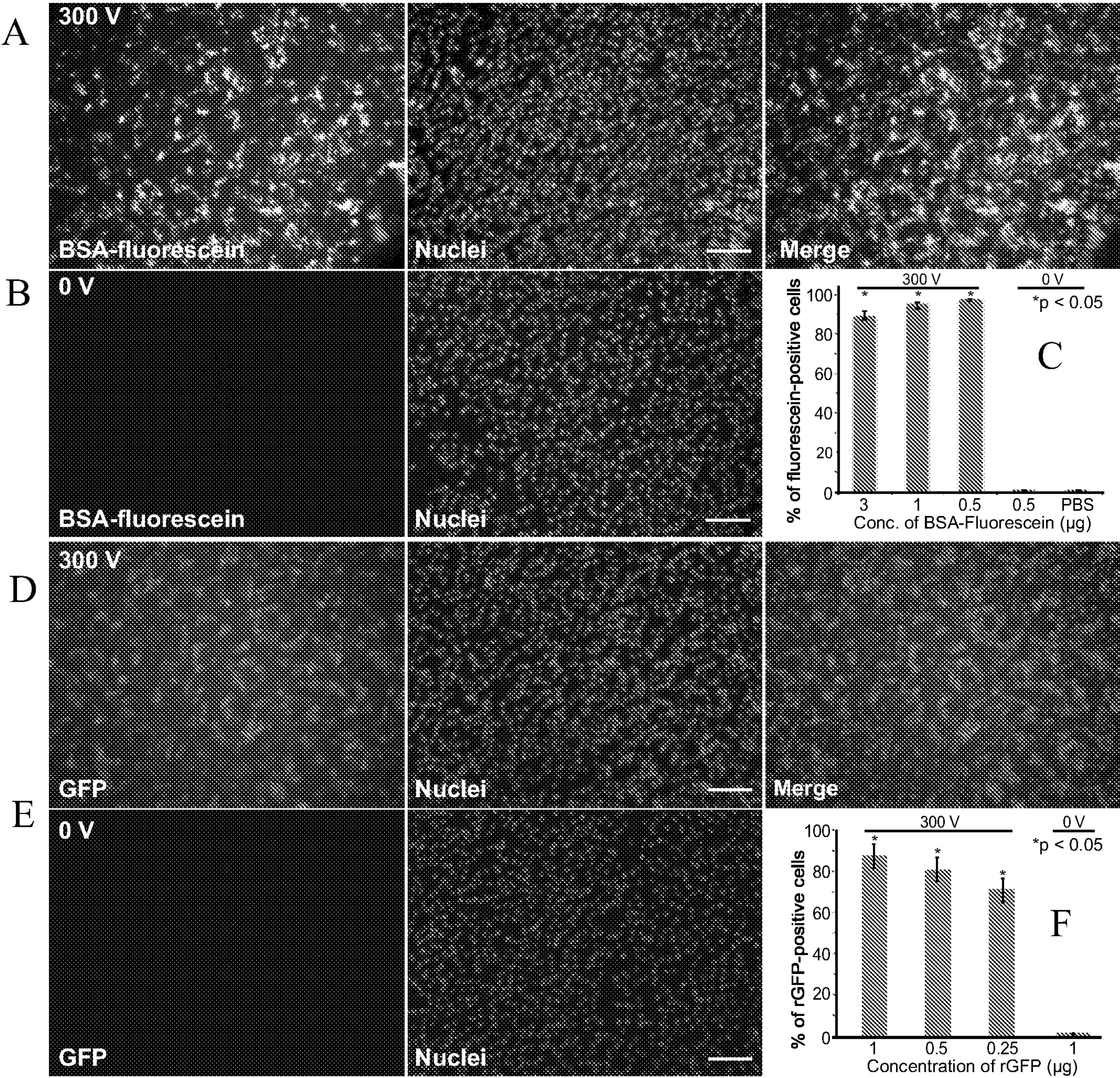
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FIGs. 8A-8B

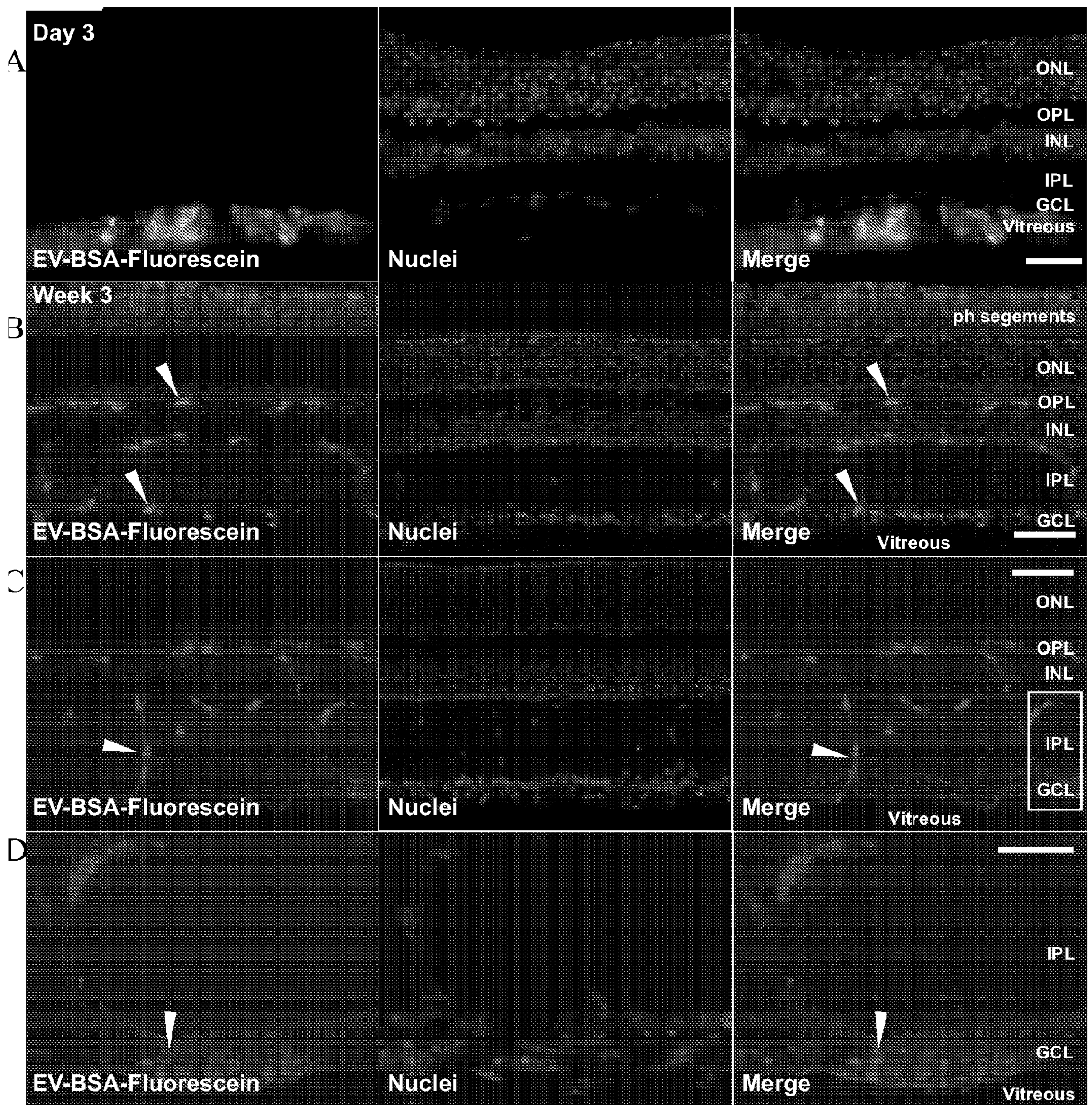


FIGs. 9A-9I

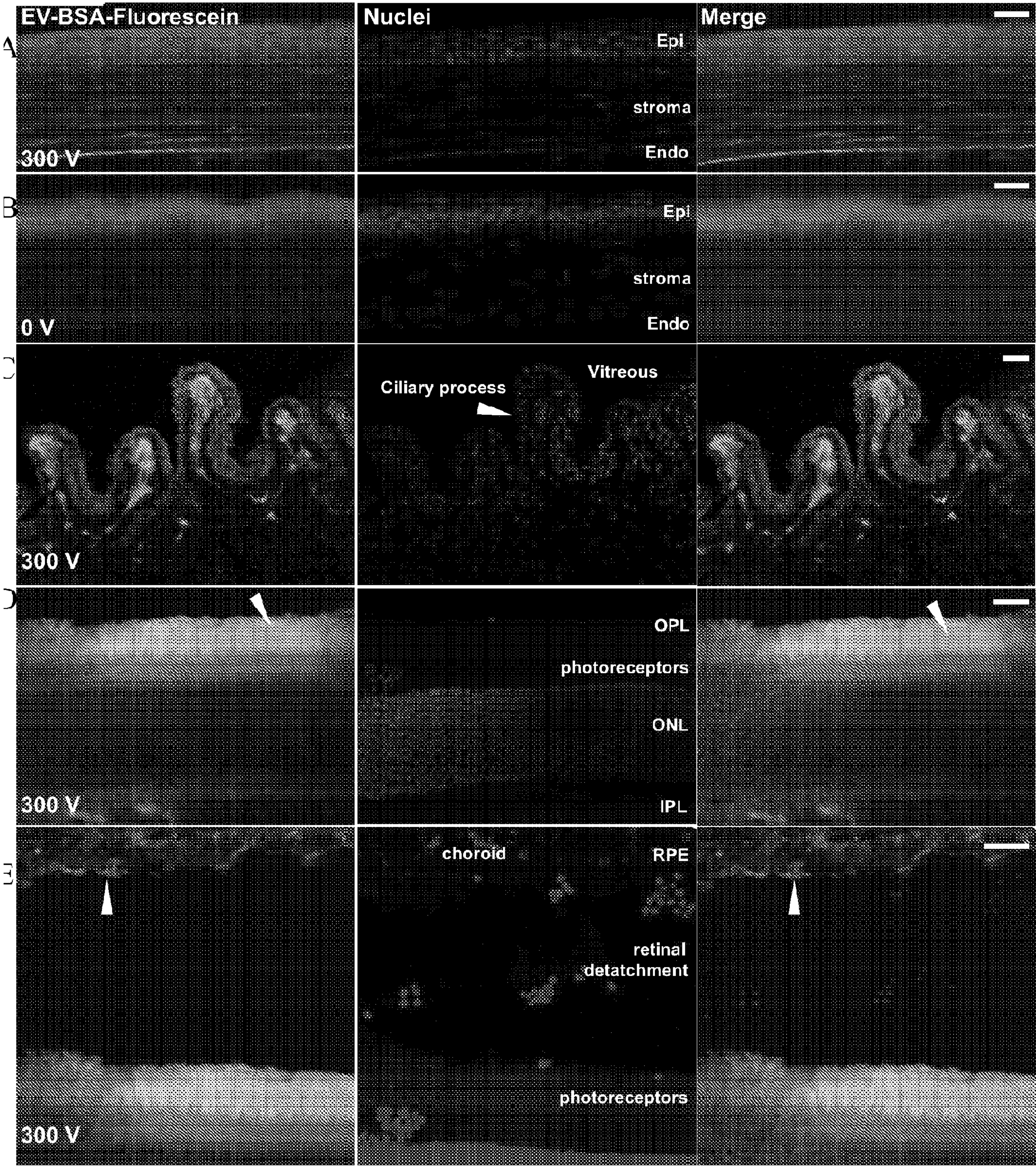


FIGs. 10A-10F

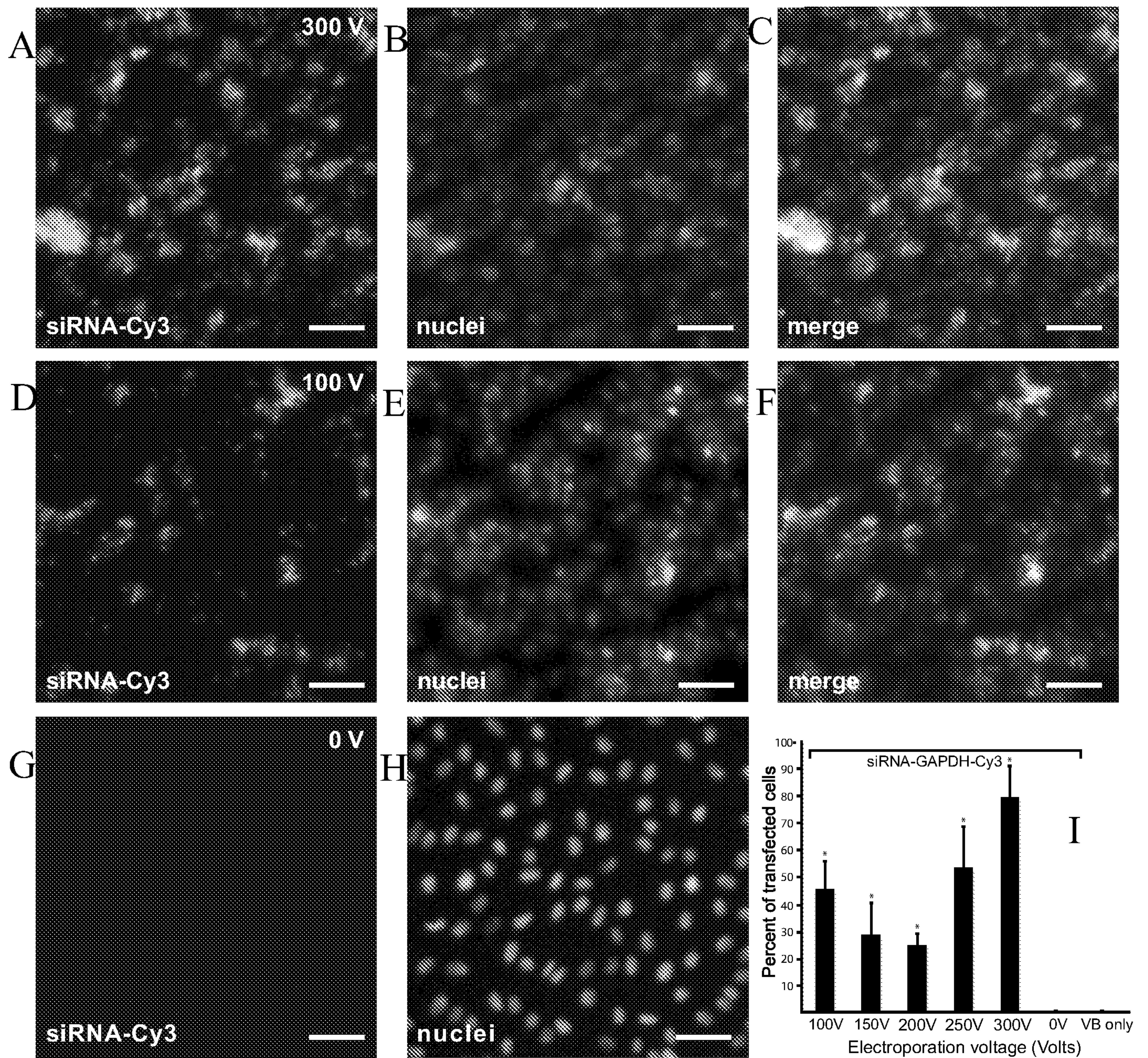
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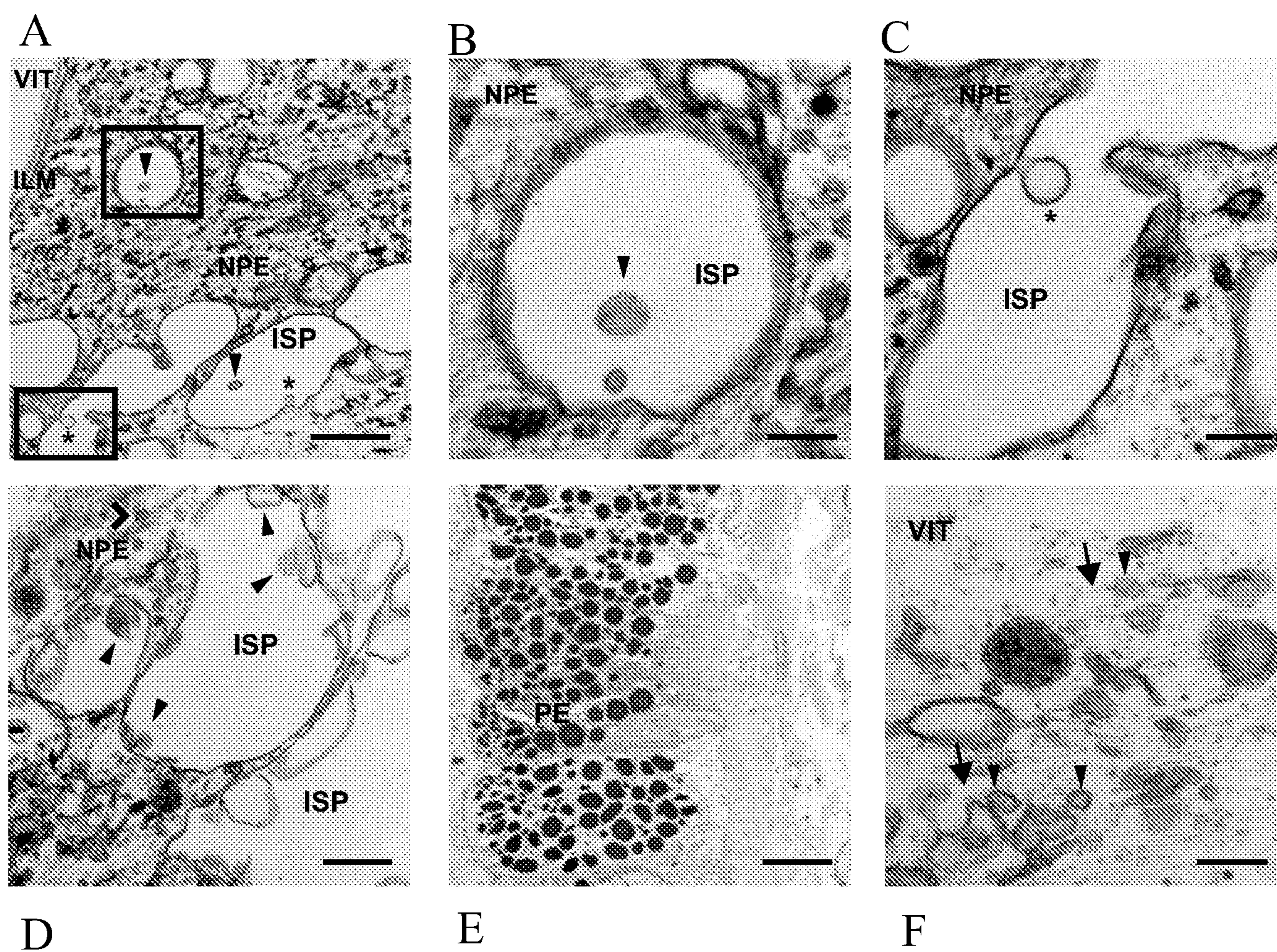
FIGs. 11A-11D



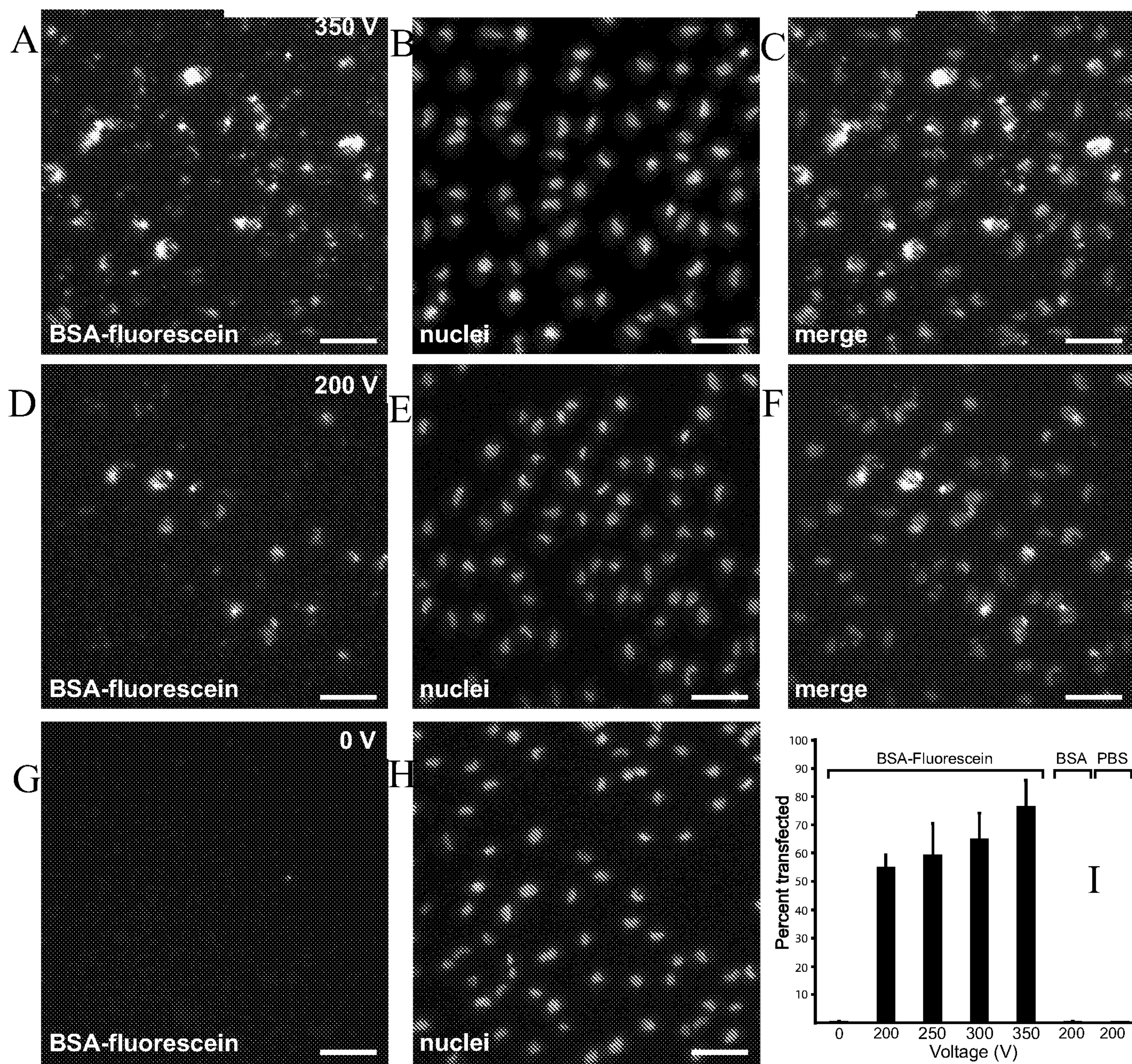
FIGs. 12A-12E



FIGs. 13A-13I

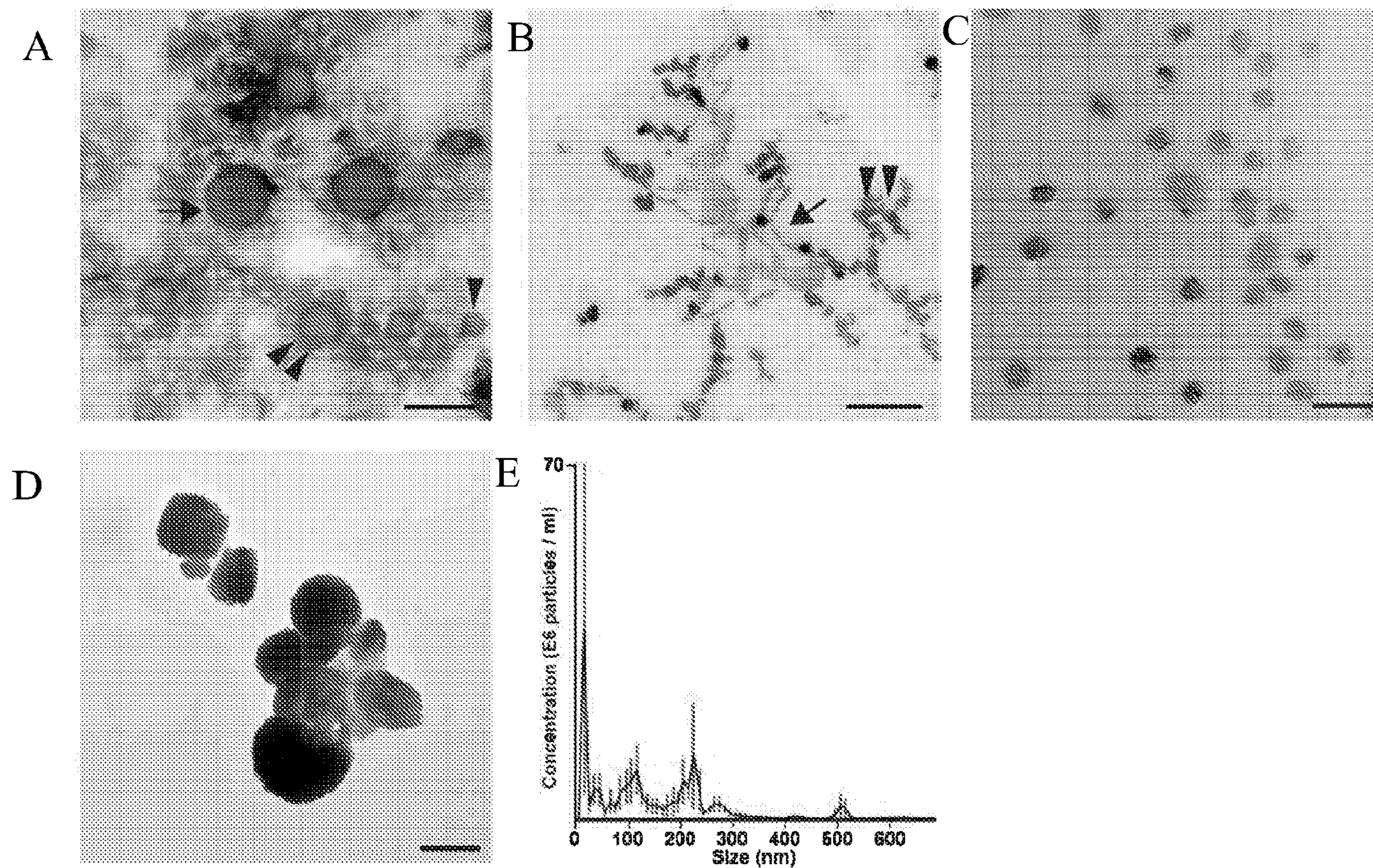


FIGs. 14A-14F

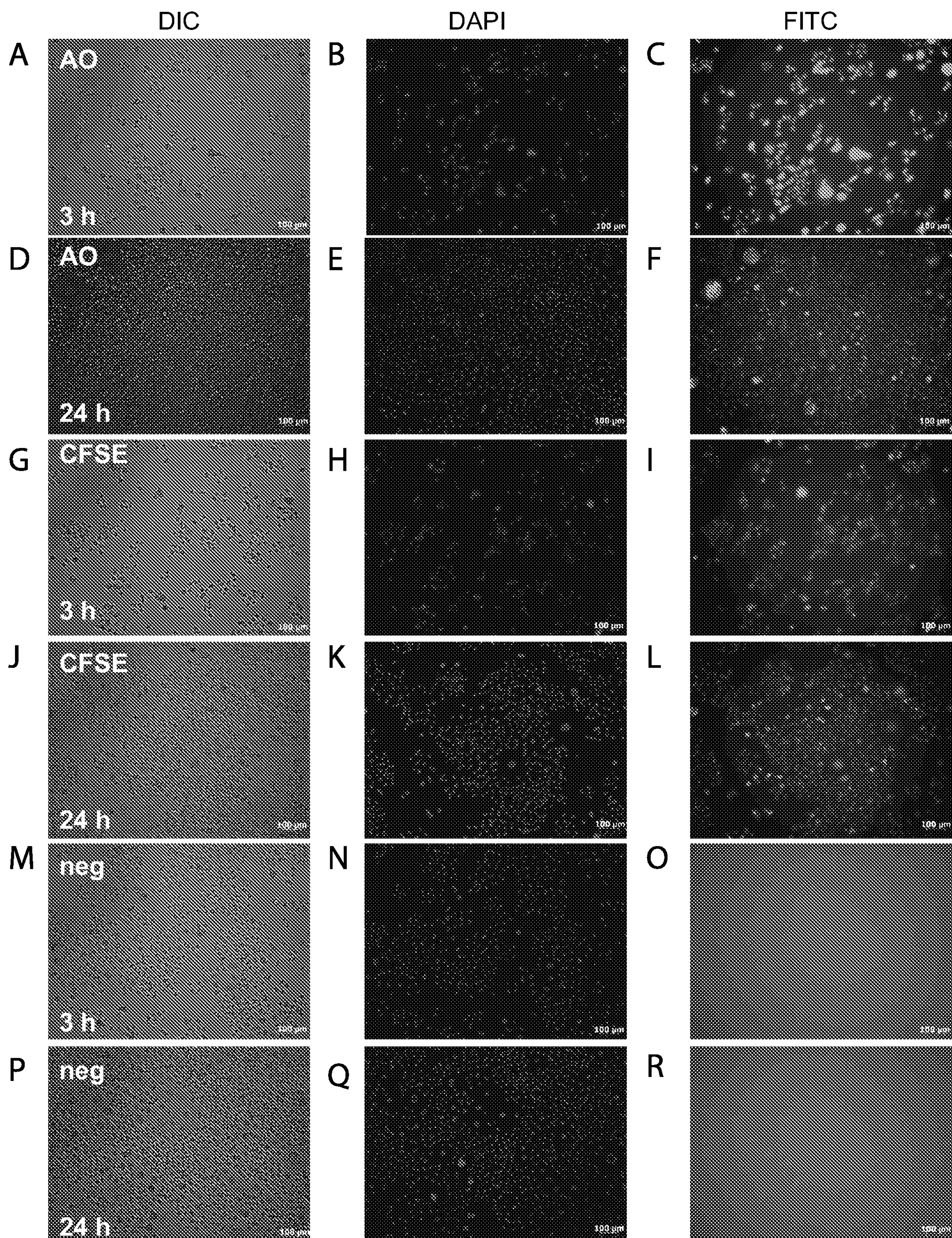


FIGs. 15A-15I

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FIGS. 16A-16E



FIGs. 17A-17R